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MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 8 – Ecological Assessment of Pollution Effects

with the cooperation of the
United Nations Environment Programme

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

UNITED NATIONS ENVIRONMENT PROGRAMME

MANUAL OF METHODS IN AQUATIC ENVIRONMENT RESEARCH

Part 8. Ecological assessment of pollution effects
(Guidelines for the FAO(GFCM)/UNEP Joint Coordinated Project on Pollution in the Mediterranean)

by

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PREPARATION OF THIS MANUAL

This Manual was prepared as part of a cooperative project of the United Nations Environment Programme entitled:

FAO(GFCM)/UNEP Joint Coordinated Project on Pollution in the Mediterranean

with the Food and Agriculture Organization of the United Nations as cooperating agency.

DEFINITION OF MARINE POLLUTION

Pollution of the marine environment means: "The introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) which results in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water and reduction of amenities".

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PREFACE

Under the Mediterranean Action Plan, developed by the United Nations Environment Programme and endorsed by the Intergovernmental Meeting on the Protection of the Mediterranean (Barcelona, 28 January - 4 February 1975), the Co-ordinated Mediterranean Pollution Monitoring and Research Programme was established. As part of this programme, an FAO(GFCM)/UNEP Joint Co-ordinated Project on Pollution in the Mediterranean was initiated. The project, among other subjects, aims at organizing a cooperative programme on Research on the effects of pollutants on marine communities and ecosystems (MED V).

The Operational Document, which serves as the programmatic basis for the collaboration of Mediterranean laboratories in the above-mentioned project, was developed at the Expert Consultation on the Joint Coordinated Project on Pollution in the Mediterranean, convened by FAO(GFCM) in Rome, 23 June - 4 July 1975. This document specifies the communities and ecosystems to be studied, as well as the parameters and effects to be analysed and the general methodology to be used.

At the request of the Mid-term Expert Consultation on the Joint FAO(GFCM)/UNEP Co-ordinated Project on Pollution in the Mediterranean, which reviewed the progress of the programme, and was held in Dubrovnik, 2-13 May 1977, this Manual was prepared to give detailed guidance on methods used in the assessment of pollution-induced modifications of marine ecosystems. Its ultimate aim is to contribute to the identification of the effects on marine life of pollutants from different sources. This will assist the Contracting Parties of the Convention for the Protection of the Mediterranean Sea against Pollution (Barcelona Convention) to take appropriate measures against adverse effects of pollution caused by dumping from ships and aircrafts, pollution from ships, pollution resulting from exploration and exploitation of the continental shelf and the sea-bed and its subsoil, and pollution from land-based sources (Articles 5, 6, 7 and 8 of the Convention). It is expected that experience gained during the use of this manual will lead to comments and proposals for improvements which should be sent to FAO. Such comments and improvements will form the basis for the preparation of reference methods for studies on pollution in the Mediterranean.

The Manual was prepared by Dr. J. Štirn in the capacity as consultant to the Centre de Recherches Océanographiques et des Pêches, Algiers, acting as Regional Activity Centre for MED V. The author expresses his thanks to scientists from the Station marine d'Endoume, Marseille, for helpful discussions, particularly to Dr. D. Bellan-Santini and Dr. M. Travers who substantially contributed to and reviewed the manuscript. Ms. M. Taylor, Paris, undertook the task of language editing. Final editing and compilation were done by the staff of the FAO Fishery Resources and Environment Division, particularly Dr. H. Naeve.

The views expressed in the Manual are those of the author and do not necessarily represent the views of either FAO or UNEP.

SUMMARY

Although the consequences of significant pollution appear in the marine environment as changes of most characteristics of the ecosystem, this manual is mainly providing methods for the assessment of pollution-induced modifications as shown in the structure, and particularly in changed diversity, of communities. The manual recommends that such investigations be focussed on benthic communities, combined with as much information as possible on pelagic environmental conditions, bioproductivity and communities. Therefore, basic methods for sampling, processing and determinations of phyto- and zooplankton are given as well. Methods for benthic investigations are described in more detail, particularly for the littoral soft-bottom communities, providing information on recommended strategy, design and execution of sampling, processing of samples, taxonomic identifications, quantitative analyses of floro-faunistic components, and biocoenotic interpretations of data obtained.

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1. INTRODUCTION

The effects of rising pollution stress upon marine environments have become a cause of increasing concern, perhaps especially because of the possible feedbacks to our own health, economic welfare and enjoyment of a healthy environment. Even when such effects are significant enough to be observed and/or measured as microbial or chemical contamination of sea water and marine food organisms, as decreased stocks of economically important biota, etc., they provide information on local rather than on overall pollution impacts. Physico-chemical measurements of pollution-induced modifications of the marine environment, i.e. deviations from "natural" conditions in sea water and on the sea bed (such as routinely analysed pH, Eh, CO_2-system, O_2, BOD, nutrients, organic load, seston and specific pollutants) if measured per se, separately from biotic systems, are similarly ineffective. Whenever we consider any form of marine pollution we must be aware of the fact that, despite the many changes it may cause in the physico-chemical properties of the water body and the sea bottom, the ultimate consequences are inevitably of a biological nature. The most critical effect of pollution is that on living organisms, and is a disorder which initiates a circulus vitiosus of secondary changes in the environment and in communities. Therefore, the investigations of ecosystems and, particularly, of their communities should constitute an important part of any marine pollution study.

Since there are, even in recent ecological literature, numerous and sometimes contradictory interpretations of the basic concepts of marine ecosystems, a short theoretical introduction seems necessary, at least in order to avoid terminological misunderstandings. For practical reasons, however, a larger part of relevant ecological theory has been incorporated into the section of the manual on the interpretation of structural features as a means of pollution-induced modifications of benthic communities (see 2.4.9) and only basic definitions are given here, following mainly the concept of Odum (1971).

1.1 The Ecosystem

Living organisms and their abiotic environment are inseparably interrelated and interact with each other. Any unit that includes all the organisms (i.e. the community) in a given area, interacting with the physical environment so that a flow of energy leads to a clearly defined trophic structure, biotic diversity and material cycles (i.e. exchange of material between living and non-living parts) within the system, is an ecosystem.

From the trophic standpoint, an ecosystem has two components, the autotrophic and the heterotrophic. The autotrophic component of an open ocean ecosystem is obviously concentrated as phytoplankton in the euphotic part of its pelagic zone, while heterotrophs more or less equally inhabit both pelagic and benthic environments. In shallow coastal waters quite an important part of autotrophic macrophyta may belong to the benthic zone, to which most heterotrophic populations also belong.

For descriptive purposes it is convenient to recognize the following components as comprising the ecosystem:

(a) inorganic substances (C, N, P, S, microelements) involved in material cycles and physical sources of energy (radiation);

(b) organic compounds that link biotic and abiotic;

(c) producers, i.e. autotrophic organisms;

(d) macroconsumers or phagotrophs, i.e. heterotrophic organisms, chiefly animals, which ingest other organisms or particulate organic matter present in marine ecosystems as:

- suspension-feeders, i.e. pelagic or benthic filtrators,
- macropredators, equally present in plankton, benthos and nekton, and
- benthic deposit-feeders;

(e) microconsumers (saprotrophs and osmotrophs), i.e. heterotrophic organisms, chiefly bacteria and fungi, which break down the complex compounds of dead protoplasm and other forms of biodegradable organic matter, absorb some of the decomposition products, and release inorganic nutrients that are usable by the producers, together with organic substances which may provide energy sources or which may be inhibitory or stimulatory to other biotic components of the ecosystem.

From the functional point of view ecosystems are characterized by structures and processes which may all be modified as consequences of pollution stresses.

1.1.1 Energy circuits

As all biological systems, ecosystems possess the essential thermodynamic characteristic of being able to create and maintain a high state of internal "order", or a condition of low entropy. This is achieved by a continual dissipation of energy of high utility (light or food) to energy of low utility (heat). In the ecosystem, "order" in terms of a complex biomass structure is maintained by the integral community respiration which continually "pumps out disorder". Thus the simplification of communities, as induced also by pollution stresses, may cause increased entropy and/or unbalanced deposits of organic matter.

The basic process in an ecosystem is its primary productivity. This is the rate at which radiant energy is stored by photosynthetic or chemosynthetic primary producers in the form of organic substances which can be used as food material for the process of heterotrophic secondary productivity of a herbivorous, a detritovorous and usually 2 to 3 carnivorous trophic levels. In accordance with the second law of thermodynamics the energy flow is increased at each trophic step by the heat loss that occurs with each transfer of energy from one form to another. Usually a large proportion, 80 to 90 percent, of the potential energy is lost as heat.

The transfer of food energy from the primary source through a series of organisms with repeated eating and being eaten is referred to as the food chains, of two basic types: the grazing food chain, from primary producers through herbivores and carnivores, and the detritus food chain, which goes from dead organic matter into micro-organisms, large detritovores and their predators. Food chains are interconnected into complex food webs.

The standing crop biomass which can be supported by a steady flow of energy in a food web depends to a considerable extent on the size of species in a community; the smaller the organisms, the greater their metabolism per unit of biomass; consequently, the smaller the organisms, the smaller the biomass which can be supported in an ecosystem. Since many types of pollution stress may cause ecosystem modifications which favour predominance of tolerant micro-organisms, the modified communities show low biomass, high metabolism and increased rates of entropy. In addition, due to depressed functions of less tolerant predators, there might be also a significant increase of dead organic matter deposited in sediments of such modified ecosystems.

1.1.2 Biotic communities

The functions of ecosystems are carried on more or less efficiently by more or less developed structures of communities. Therefore, communities not only have a definite functional unit with characteristic trophic structures and patterns of energy flow but they also have compositional unity in that it is probable that certain species will occur together. However, species are to a large extent repleaceable in time and space so that functionally similar communities may have different species compositions.

Not all organisms in the community are equally important in determining the nature and function of the whole community. A relatively few species or species groups in a community (the so-called ecological dominants) generally exert the major controlling influences upon energy flow and strongly affect the environment of all other species by virtue of their abundance, size, production or other activities. The degree to which dominance is concentrated in one, several or many species can be expressed by an appropriate index of dominance that sums the importance of each species in relation to the community as a whole.

While the few dominant species largely account for the energy flow in each trophic group, it is the large number of rare species that largely determine the species diversity of trophic groups and whole communities. Ratios between the number of species and values of abundance, biomass or productivity of individuals are called species diversity indices. Species diversity tends to be low in physically controlled ecosystems (i.e. subjected to strong physico-chemical limiting factors, including pollution stresses) and high in biologically controlled ecosystems, and is directly correlated with the stability of ecosystems.

1.1.3 Development, evolution and homeostasis of ecosystems

The development of an ecosystem, i.e. its ecological succession, may be defined in terms of the following parameters:

(a) It is an orderly process of community development that involves changes in species structure and community processes with time; it is reasonably directional and therefore predictable.

(b) It results from modification of the physical environment by the community; that is, succession is community-controlled even though the physical environment determines the pattern and the rate of change, and often sets limits as to how far development can go.

(c) It culminates in a stabilized ecosystem in which maximum biomass, diversity and symbiotic functions between organisms are maintained per unit of available energy flow.

The whole sequence of communities that replace one another in a given area is called the seres; the relatively transitory communities are seral stages, while the terminal stabilized system is known as the climax. The importance of ecologic successions is, up to a climax equilibrium, the increase in control of, or homeostasis with, the physical environment in the sense of achieving maximum protection from its perturbations. In contrast to developmental or other unstable communities, such as those under pollution stresses, in climax communities the production nearly equals the total respiration and there is no net annual accumulation of organic matter.

As a consequence of a long evolutionary history, and because of the typical stability of the marine environment, its ecosystems and a great majority of communities are in a climax stage. Exceptions are estuaries, lagoons and some upwelling zones which may be inhabited by less stable communities due to fluctuations of natural physical conditions, as well as an increasing number of coastal zones which are also a target of pollution stresses. Under pollution stresses, the previously climax communities can undergo a regressive evolution, leading into the most "immature" stages of an unstable ecosystem, as described below for benthic communities (see 2.4.9).

1.2 Pollution

Regardless of consequences such as microbial contamination of recreational waters and sea food, accumulation of persistent toxicants in sea food and aesthetic degradation of coastal zones, and purely from an ecological standpoint, pollutants can be classified as follows:

1.2.1 Toxic or inhibitory pollutants

As a consequence of the lethal or subtle effects of these pollutants upon physiological functions, behaviour, nutrition, reproduction, metamorphosis and genetics of marine biota, all or some species populations may disappear from an ecosystem, or may cause regressive modifications in an ecosystem. The most common pollution with these effects is caused by:

- thermal effluents;
- non-biotic microelements such as Cr, Ni, Hg, Cd, Ag, Pb, Al, Ti, and free chlorine, cyanides and elementary P;

- excess levels of biological microelements such as Fe, Mn, Zn, Cu and Mo;
- some components of mineral oils and their derivates;
- phenols, detergents, chlorinated hydrocarbons and some other synthetic organic substances;
- excess levels of intermediate products of decomposing organic matter, nitrites, ammonia, and H_2S;
- biodegradable organic matter itself if causing high BOD and, consequently, a significant decrease of dissolved oxygen;
- high levels of strong acids or alkalies shifting normal pH range of sea water.

1.2.2 Enrichment pollutants

Any type of effluents or runoff which increase natural levels of nutrients for primary producers, for example ammonia, nitrites, nitrates, phosphates, possibly limiting microelements such as Fe, biostimulatory organic substances (vitamins, phytohormones) and chelators, are a potential cause of relative eutrophication (see 2.4.9 below). These are:

- crude and treated sewage, including detergents;
- effluents from the food-processing industry;
- farm waste waters;
- runoff from fertilized agricultural areas;
- urban runoff;
- polluted atmospheric water and fallout.

The most important source of enrichment pollutants is, however, river discharges, which contain all these pollutants as well as naturally eroded nutrients.

1.2.3 Inert suspended solids

Although direct physiological effects of increased levels of suspended matter, such as clogging of gills and filtration organs, might rarely reach significant levels, their indirect consequences, such as reduction of light penetration, blanketing bottom substrates and adsorption functions, can induce important modifications of the marine environment and communities, particularly of the benthos. The main sources of these pollutants are:

- dumping of sewage sludge from sewerage systems and treatment stations;
- dredging spoil;
- coastal or submarine mining operations;
- industrial dumping such as waste from titanium, china clay, cement and wood-paper processing.

1.3 Ecological Assessment of Pollution Effects in Marine Ecosystems

As already mentioned, ecological methods for the assessment of pollution effects provide a greater potential for valid interpretations than physico-chemical environmental measurements or bacteriological tracers because it is possible to obtain records of prevailing conditions over long periods of time and they are relatively insensitive to temporary fluctuations in the basic environment and input rates and composition of pollutants. Environmental measurements of traceable pollutants and of natural conditions cannot be omitted, of course, and they should present an integral part of ecological pollution assessment.

The theoretical basis for the ecological approach is the fact that any significant introduction or removal of any substance or energy which is not inert to biological processes in an ecosystem must cause changes in its structure, dynamics and energy flow. In this context, it is quite irrelevent

whether the primary effect is of a toxic-inhibitory or enriching nature. In both cases the primarily modified ecosystem responds to stress by adjusting the dynamics equilibrium among its components. Depending upon the type, strength and extent of a stress factor, the ecosystem will react, due to its homeostasis, to either re-establish the previous equilibrium or establish a new one, or it shall remain for longer periods in disequilibrium. Except for the first mentioned, fully reversible situation, all ecosystem modifications must theoretically be retraceable in the structures and functions of communities. Therefore, in principle, an ecological assessment of pollution effects can be carried out by multiple or separate analyses of the following ecosystem or community characteristics:

(i) energy circuits and production-respiration and food web;

(ii) dynamics of dominant species;

(iii) composition of community and its species diversity.

Although (i) and (ii) may ultimately provide interpretations on processes involved in pollution-induced ecosystem modifications, these approaches are not practicable on account of the technical and intellectual requirements. Therefore, the methods recommended in this manual will be focussed on the purely biocoenological approach for the relevant investigations of communities.

SUMMARY

The significant introduction or removal of energy or any substance involved in biological processes inevitably induces ecosystem modifications. Therefore, the consequences of pollution appear as changes in the ecosystem's energy circuits and food webs, as well as in population dynamics and the structure and diversity of its communities.

Although any of these ecosystem modifications can be considered as a theoretical basis for the ecological assessment of pollution, investigations of community structure and diversity are of first importance. If properly studied, communities provide records of prevailing environmental conditions over long periods of time which are, in contrast with physico-chemical analyses, relatively insensitive to temporary fluctuations of the basic environment as well as of rates and composition of pollutants.

For obvious reasons, such as frequently changing community composition due to dynamics of water masses, fast growth of populations, patch distribution, etc., and the expressed seasonal dynamics and successions of most community components, the study of pelagic communities for the above purposes is rather problematic. It is recommended, therefore, that investigations be focussed on benthic communities, combined with as much information as possible on pelagic environment conditions, bioproductivity and communities.

2. ASSESSMENT OF POLLUTION-INDUCED ECOSYSTEM MODIFICATIONS BY BENTHIC INVESTIGATIONS

2.1 Mediterranean Zonation

Benthos is terminologically considered here as that part of the marine ecosystem inhabited by benthic communities, composed of organisms which live in, or closely associated with, the sea bed. Although benthic communities are present all over the bottom of the oceans, including the deepest - the abyssal (3000 to 6000 m) and hadal (6000 to 12000 m) - zones, for practical reasons only shallow-water benthos of the continental shelf will be considered here. Even within the benthos of the continental shelf only its shallowest littoral zone (from the surface to 150 to 200 m) seems to be of great importance as far as pollution impacts are concerned, although there might also be a need for information about conditions in the bathyal zone (200 to 1000 m or more), particularly in the Mediterranean Sea where the continental shelf is usually quite narrow or totally missing.

The littoral zone here corresponds to those parts of a benthic subecosystem whose submarine illumination is sufficient to support at least some benthic primary producers, in contrast to bathyal or deeper zones which are devoid of autochthonous autotrophic biota. According to the terminology introduced by Pérès and Picard (1958) and widely accepted by Mediterranean ecologists, the vertical zonation of the littoral includes the following quite distinct zones:

(i) Supralittoral - the uppermost zone above the highest tidal sea level where the sea water supply is due to waves and spray. The vertical extension of this zone is narrow in sheltered places (30 to 50 cm) and large (up to 5 m) at exposed shores. It is inhabitated by communities composed of a small number of super-tolerant species. On rocky shores these are mainly epilithic or endolithic blue-green algae, lichens, chthamalid cirripeds and littorinas. The supralittoral sandy and muddy beaches are inhabited by some "specialist" species, mainly burrowing amphipods and isopods, insects and arachnids.

(ii) Mediolittoral - corresponding to the common oceanic intertidal zone, which in the Mediterranean Sea is typically developed only in the areas with regular tides (Gulf of Gabes, upper Adriatic) with an average vertical amplitude of 80 cm. On most of the shore of the Mediterranean Sea the tidal oscillations are extremely small and therefore the vertical amplitudes of the mediolittoral are usually quite narrow, less than 40 cm. The species compositions of intertidal communities are quite different for various parts of the Mediterranean, so is their inframediolittoral microzonation (see Peres, 1967). The major components are calcareous and soft red algae, some brown and green algae, intertidal species of molluscs and cirripeds. Mediolittoral communities on soft, mainly sandy substrates are quite poor, in contrast, both in diversity of species (mainly some burrowing polychaetes and amphipods) and in population density.

(iii) Infralittoral - defined by Pérès (1967) as the vertical extent of the benthic domain, which is compatible with the existence of marine phanerogams (mainly Posidonia and Cymodocea) on soft substrates and photophilic brown algae, growing mainly on hard bottoms. Besides dominating populations of sea plants, the infralittoral is densely populated by rich benthic fauna belonging to almost all taxonomic classes of marine invertebrates and by important stocks of shallow-water fish such as labrids, serranids and sparids. Since the major limiting factor of the extension and microzonation of the littoral zone is the submarine illumination, its vertical delimitations are quite variable, corresponding to turbidity and light extinction, they extend from the lower mediolittoral to a depth of 15 m in extremely neritic, turbid and highly productive areas, and down to 50 m in typically oligotrophic Mediterranean environments. The infralittoral is the most productive subsystem of all Mediterranean Sea ecosystems. It is also characterized by extraordinarily diverse communities at all trophic levels. Unfortunately, it also presents a target for any pollution impacts. In contrast to other littoral zones, the remarkable seasonal and long-term dynamics and fluctuations in productivity and structure of infralittoral communities must be considered among their leading characteristics. The implication of this fact on research and interpretation of relevant ecological investigations of infralittoral communities should be taken into account.

(iv) Circalittoral - extending from the lowest level where the phanerogams or photophilic algae are able to live, to the maximum depth compatible with the existence of algae which can live even under the most feeble conditions of illumination (sciaphilous algae). In fact, these algae, at least the multicellular ones, may be missing because there is no adequate substrate, such as on sandy or muddy bottoms free of larger mineral particles or biogenic partial substrates. However, it is always possible to find a "lateral" correspondence which allows recognition of such biotopes, for example when a rocky substrate exists, with sessile algae and animals, at the same depth as the soft bottom which is to be compared (see Pérès, 1967). Circalittoral communities on hard substrates (most common are the so-called

coralligenous biocoenoses) are, in contrast to the infralittoral, dominated by sedentary, mainly filter feeders (sponges, coelenterates, serpulids and bryozoans), but the assemblages of sciaphilous macroalgae (mainly rhodophyceans and green siphonales) also present an important element.

Particular attention must be devoted to circalittoral communities which are developed on soft substrates made up mainly of finer fractions of terrigenous, including fluvial, sediments and sometimes coarse mineral particles which are predominantly biogenic remnants (shells, etc.). The composition of circalittoral soft substrates and their vertical distributions in the Mediterranean Sea are extraordinarily complex and variable, as also are the communities. Therefore, users of this manual should consult specific literature such as Pérès and Picard (1958) and Pérès (1967) in order to become familiar with the circalittoral benthic domain, which generally dominates the largest part of the continental shelf (see Figure 1). These communities are in most cases very diverse in both taxonomic composition and trophic structure. Although relevant relationships in Mediterranean circalittoral subsystems are not very well known, it can be stated that there is an equal representation of secondary producers (infra-and episubstratal active and passive filtrators, i.e. suspension feeders and mainly infrasubstratal deposit feeders) and tertiary producers (mainly epifaunal predatory invertebrates and demersal fish) at least as measured by standing crops. However, the benthic primary producers, which can be represented by some sciaphilous macroalgae only on coarse detritic bottoms offering "micro-hard substrates" and by diatom-films over sedimentary surfaces anywhere, are subordinated in this subsystem even more than in hard-bottom circalittoral communities. Therefore, the investigations of soft-bottom circalittoral communities should always be focused on the assemblages of leading components, which are molluscs, polychaetes, crustaceans, echinoderms and demersal fish, except in the cases of secondarily developed hard bottoms (large-size detritus, lithothamnia) with prevailing sedentary assemblages of coralligen enclaves, ascidian or bryozoan facies, etc.

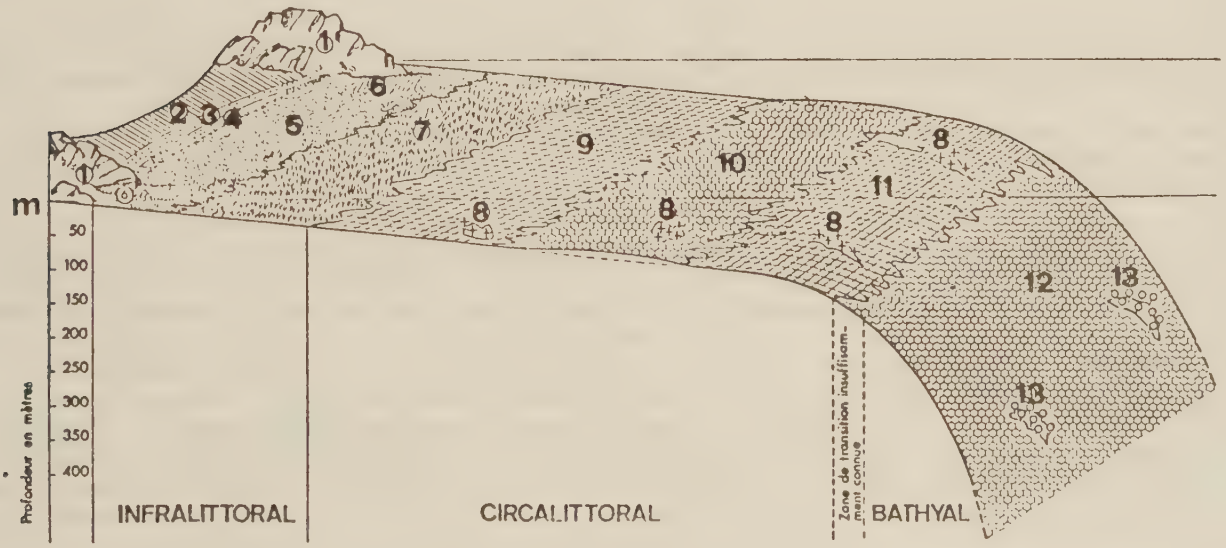


Figure 1. An example of the distribution of typical benthic communities in the Mediterranean (French coast): (1) rocky points; (2) alluvial area; (3) high and middle beach (supralittoral and mediolittoral sandy biocoenoses); (4) biocoenosis of the fine sands in very shallow waters; (5) biocoenosis of the well-sorted sands; (6) biocoenosis of the photophilous algae on rocky substrates; (7) biocoenosis of the Posidonia meadows; (8) coralligenous biocoenosis; (9) biocoenosis of the coastal detritic; (10) biocoenosis of the terrigenous mud; (11) biocoenosis of the shelf-edge detritic; (12) biocoenosis of the bathyal mud; (13) biocoenosis of the deep sea corals
(From Pérès, 1967, with kind permission of George Allen and Unwin Ltd., London. © 1967).

The bathyal zone, as a transition between the euphotic littoral and abyssal domain, presents a widely distributed subsystem in the Mediterranean, where shelfless areas are quite common, yet from the standpoint of pollution-induced modification it seems rather doubtful that such effects can be detected in the structures of bathyal communities, at least at the present levels of pollution loads. In addition, the bathyal fauna are generally rather sparse and quantitative sampling present a number of technical problems. Therefore, bathyal investigations should not generally be recommended, although some long-term observations at selected localities in the Mediterranean might provide interesting indications.

2.2 Types of Benthic Biota and their Identification

From a taxonomical point of view almost all classes of lower plants and invertebrates take part in benthic communities (exceptions are some purely holoplanktonic groups such as flagellates, siphonophores, ctenophores, pelagic tunicates, etc.), and the majority of them are found living together in all types of habitats and zones, except in the supralittoral and mediolittoral zones. In any benthic investigations a team of skilled taxonomists is required for the identification of the most important classes, and access to collaborating specialists elsewhere for the taxonomic identification of that part of the remaining biota which is essential for an adequate knowledge and interpretation of community structures. Although there is no universal scheme which can advise on crucially important taxonomic groups to be identified at species level, some basic suggestions derived from practical experience might be helpful. They are given below, according to types of benthic assemblages, classified artificially for practical purposes.

2.2.1 Macrobenthos

For practical reasons all the larger organisms, i.e. those retained by sieves of 1.0-mm mesh, are referred to here as the macrobenthos, although this definition does not fit exactly into the benthic classification as approved by CIESMM (Pêrês, 1965), which divides macrobenthos into megisto-, mega- and mixobenthos. The most common macrobenthic biocoenotic complexes in the Mediterranean are the following:

Supralittoral and mediolittoral macrobiota on hard substrates are comprised of a rather small number of widely known intertidal species. Therefore, a qualified ecologist who is not necessarily a specialist in taxonomy can identify most species, especially if the flora and fauna of the investigated area have been studied previously by specialists and reference collections are available. Sampling can be performed manually by the simple square method, accompanied if possible by photogrammetric records (see 2.4.2)

Infralittoral and circalittoral macrobiota on hard substrates and on secondary enclaves within detritic sedimentary bottoms are characterized by the highest level of diversity and complexity. Sampling is most difficult, almost unfeasible without diving techniques (see 2.4), The basic structure of communities can be described and quantified only on condition that the taxonomic groups are properly identified. Therefore, the collaboration of specialists for the following taxa is essential: macroalgae, sponges, anthozoans, polychaetes, amphipods, bryozoans, ascidians. The most practical solution is at least to have specialists for macroalgae, polychaetes and amphipods in the operating team; non-specialist members can take care of identifications of other critical groups (sipunculids, molluscs, decapods, pantopods and echinoderms) provided the fauna has been studied previously by specialists and literature and reference collections are locally available. For the identification of sponges, bryozoans and ascidians consultant specialists have to be found.

Macrobiota of infralittoral phanerogam communities, actually prairies of sea grasses (Posidonia, Cymodocea and Zostera) are less diverse but very productive and quite consistent in their physical structure. Therefore, sampling on deeper bottoms causes quite difficult problems which can successfully be avoided only by diving methods; however, at the upper infralittoral level sampling can be performed manually by some practical and simple equipment (see 2.4.3). The basic community structures can be defined by identification of the following taxonomic groups:

(a) Phanerogams and dominating epiphytic macroalgae, molluscs, decapods, mysids and echinoderms, which can be handled by non-specialist ecologists,

(b) Polychaetes and amphipods, whose identification obviously requires specialists, ideally as members of operating research teams.

Littoral and bathyal macrobiota on free sediment bottoms develop entirely different benthic communities on soft substrates at various levels of littoral and bathyal zonation (see 2.1), as well as in relation with different types of sediments and with environmental conditions of adjacent water masses. However, their composition by major taxonomic groups is more or less similar. There are of course some important exceptions. In the mediolittoral and upper infralittoral zone some groups are missing which are normally present in deeper littoral zones and in bathyal communities. On the other hand, some groups whose main distribution is within bathyal and abyssal domains, such as hexantillid sponges, milleporid hydrozoans, most brachiopods and pogonophoras, are missing from littoral communities. However, deeper zones are devoid of any autotrophic algae. Since these differences are of minor importance as far as the strategy applied to the investigations of the basic structures of communities is concerned, some common rules may be useful. Sampling can be performed in the mediolittoral and upper infralittoral zones manually; in all deeper zones, however, grabs, anchor dredges or diver-operated suction samplers should be used for quantitative sampling, and various dredges, trawls, etc., for qualitative sampling (see 2.4.1). Among critically important taxonomic groups most identifications of anthozoans, molluscs, decapods, echinoderms and fish can be performed by non-specialist but skilled ecologists of the operating team. For the macroalgae, polychaetes and amphipods, the team should have its own specialists; for sponges, isopodes, mysids, ascidians and some less important groups collaborating specialists have to be found elsewhere.

2.2.2 Meiobenthos

Following the classification adopted by CIESMM (Pérès, 1965), this term refers to all benthic organisms which pass through a 1.0-mm sieve, but are retained by a mesh of 0.1 mm. It is, however, more practical to set the lower inferior limit at 0.06 mm, which is the mesh size of the finest sieves meiobenthologists normally use.

Organisms passing such fine sieves are known as microbenthos in sensu stricto, composed mainly of microbes, diatoms and protozoans. Although the study of microbenthos would certainly contribute important information on ecosystem conditions, particularly for pollution-oriented investigations, it requires specific methods. Therefore, the microbenthos shall not be considered in this manual.

By its universal distribution, the meiobenthos is found in practically all marine biotopes, also within the microspace made by dense populations of algae and sedentary fauna on hard bottoms, but only the soft bottom, i.e. mud and interstitial sand meiobenthos, will be considered here. The interstitial meiobenthos, or meiofauna in its classical meaning, inhabits the pore spaces of sandy sediments where it moves without disturbing the substrate; the mud meiofauna, on the other hand, makes passages through it and is therefore found more densely only near the surface of sediment layers. Since the sampling methods for both types are quite similar they can be considered together.

For obvious reasons the meiobenthos is composed mainly of animals belonging to practically all classes of metazoans, while the protozoans are represented by their largest forms, e.g. Foraminifera and Ciliata. Gnathostomulida, Gastrotricha, Kinorhyncha and Tardigrada are exclusively meiobenthic although they are not the most abundant. Nematoda, Harpacticoida, Turbellaria, Oligochaeta and smaller species or juveniles of Polychaeta are more abundant. Quantitative sampling of soft-bottom meiobenthos is usually done by coring techniques or by taking core subsamples from grab or other large-size benthic samples (see 2.4.3).

Taxonomic identifications of meiobenthic biota can be performed only by specialists, even the sorting into major taxonomic groups might be problematic for non-specialist research teams. Although the information on meiobenthic conditions is extremely important, particularly for pollution-oriented ecological studies, it seems impractical to consider the relevant investigations as part of such studies since at present only a few laboratories in the Mediterranean area can deal with meiobenthic biota at species level. However, analyses of meiobenthic biomass, total abundance and its division into dominant taxonomic groups can be made, and the selected methods are included in this manual.

SUMMARY

Since marine pollution usually affects rather localized coastal marine environments, it is sufficient in most cases to investigate benthic communities of the littoral, i.e. euphotic, zone only. For the purpose of ordinary assessment, it is recommended that investigations be focussed on macrobenthic communities because their floro-faunistic composition is usually better known and they are, from the biological point of view, more convenient to work with than the meiobenthos. Any information which can be obtained on meiofauna is, of course, welcome.

The most important communities to be studied are the intertidal and upper-infralittoral of hard bottoms, seagrass prairies and infra-circalittoral communities on soft bottoms. When selecting communities to be investigated, particular attention must be given to comparative studies of similar assemblages in a non-polluted region of the same area, as such parallel investigations constitute the most adequate approach to reliable assessment.

Although the zones to be studied have been restricted to the littoral, their communities are composed of almost all classes of lower plants and invertebrates, which have to be identified at species level. Some minor and morphologically distinct groups can be identified by non-specialist ecologists, but skilled taxonomists are required for algae, molluscs, polychaetes and amphipods. Since exact taxonomic identifications are a prerequisite for reliable analyses of communities, it is recommended that specialists for these groups are part of the research teams responsible for assessment projects. Updated taxonomic literature, reference collections of local biota, and collaboration for taxonomic groups not mentioned above, are also essential.

2.3 Basic Strategy of Benthic Investigations and Design of Sampling Programmes

The strategy of pollution-oriented benthic investigations will obviously depend on many factors, such as:

- specific conditions of the investigated area, its marine habitats, communities etc.
- types of existing or potential pollution impacts and spatial extent of pollution;
- stage of existing floro-faunistic and ecological knowledge of the area to be investigated;
- available facilities in staff, ships, gear, etc.;
- desired levels of ecological interpretation of required data.

As there are almost unlimited variations in such research programmes, it would be impossible to establish standards or general rules. However, some basic principles and experiences are given here to assist those who approach sampling design for the first time, and also as a contribution towards improved uniformity of benthic investigations and comparability of results.

2.3.1 Selection of investigation areas

The selection of areas to be investigated for assessment of pollution impact upon benthic communities should generally correspond to the following principles:

- the outer limits of the area should reach the "natural" environments which are in no circumstances influenced by local pollution sources;
- if possible, this extension of the investigated area should refer both to radial directions from the centre of the pollution source towards offshore bottoms and left-right laterally along the shores;
- as many types of benthic communities as possible should be present within the investigated area, assuming, of course, that suitable research teams are available;

- besides the investigated area which is polluted or likely to be polluted in the future, it is most advisable to select, for the purposes of parallel investigations, an unpolluted area which is as similar as possible in habitats and communities.

2.3.2 Acquisition of data and preliminary investigations

The design of programmes for quantitative and representative sampling of benthos is always a difficult task, and almost unfeasible without background information. The more information there is on a selected area before planning, the better the sampling programmes and their results. The following information is needed for proper planning of quantitative benthic studies.

(i) Bathymetric and geomorphological data for the investigated area available from existing documents, compiled into a basic map of the area. If such a compilation is not adequate for the presentation of major geomorphological formations of the submersal coastal slopes and of the plain sea bottom, additional echo-soundings along critical transects in deeper waters and orientative mapping by divers for coastal hard bottoms should be done.

(ii) Sedimentological data from all available sources, including navigation charts, plotted in the form of convenient histograms on the basic map. By simple interpolations a map of the topographical distribution of the major sedimentological types within the investigated area can be made. This information, combined with the knowledge on the distributions of distinct types of water masses within the investigated area constitutes one of the most important elements in planning quantitative sampling programmes. Therefore, it is advisable to complete sedimentological studies of the investigated area (if such data are not already available) before the final setting-up of benthic sampling programmes, although both samplings are usually carrried out at the same time for practical and economic reasons.

(iii) Oceanographic data on distribution of water masses and their movements as well as the trophic conditions in the pelagic environment of investigated areas. These data are essential.

(iv) Spatial distribution of pollutants within the investigated area. This has to be known in order to select a suitable area and to identify its extension and limits. In most cases such information is provided by the data on spatial distribution of coliforms and detergents, which are for the time being still the most practical tracers for the marine distribution of sewage and for the majority of mixed industrial effluents. There are of course industrial effluents which do not contain these tracers, and the detection of effluent distributions might be quite difficult, requiring sophisticated analyses of specific substances. The most useful information generally and for the latter cases in particular, is provided by a fair knowledge of prevailing currents and other movements of water masses within the investigated area, indicating most probable distribution of pollutants.

(v) Qualitative data on the types of benthic communities and their biota. These data alone or together with the above information form the basis for the design of sampling programmes. Therefore all existing information should be compiled and brought up to date by preliminary benthic investigations, carried out by qualitative dredging on soft bottoms and by direct diving observations and collecting on hard bottoms. Observations and underwater photos made by divers are also extremely useful. The divers can be replaced by underwater television equipment, if available, and particularly for deeper bottoms (over 50 m).

2.3.3 Final design of a benthic sampling programme

As a result of the preparatory work and preliminary investigations described above, the selected area can be topographically plotted into major patches inhabited by various types of benthic communities, such as hard-bottom littoral communities, prairies of sea grasses, shallow-water sandy or muddy assemblages, and various types of deeper soft-bottom assemblages developed on distinct sedimentary substrates (marl, sand, mud, etc.). With such a basis, the following design procedure is recommended:

(1) Layout of sampling stations for homogeneous benthic assemblages

For typical soft-bottom communities in deeper waters, seagrass prairies or similarly homogeneous formations, the grid is made up of a number of uniformly located stations covering each of the identified patches within the selected area. The number of stations per patch depends firstly on the homogeneity of the relevant communities judged roughly on the basis of preliminary investigations. Obviously, high rates of biotic homogeneity require a smaller number of stations and vice versa. Considering natural distributions, which are usually aggregated, rarely random, and only in specific conditions about uniform, the higher number of stations per patch the better and more representative will be the results of sampling. In practice, however, the number of stations tends to be a compromise between the desirable and the technically feasible. Therefore, in the preparation of station grids careful consideration must be given to all local and specific conditions. Particular attention should be paid to the possible existence of different water masses with different trophic conditions, pollution loads, etc., which of course make quite different "pelagic ceilings" above seemingly homogeneous benthic communities. In such cases the cover of stations at the variable transition zones (frontal fields between different water masses) must be correspondingly denser.

(2) Layout of sampling stations for gradient benthic environment

For shore littoral communities, both on hard and sedimentary bottoms which have an obvious vertical zonation, the sampling stations are determined along transects from the shore to the beginning of a patch on steady, deeper soft bottoms or seagrass prairies where the uniform station grid is determined as above. In considering the number of stations required per transect, the same principle is applicable; however, the distribution of stations along transects in this case cannot be uniform. Since the zonaton of communities is more tight in surface layers of the supralittoral, mediolittoral and upper infralittoral, the distribution of sampling stations along the shallow part of the transect must obviously be denser than in deeper layers of more homogeneous phytal and circalittoral communities.

A similar approach with transects is suggested for those parts of investigated areas where there are significant gradients of environment stress factors. Natural examples of such formations are estuaries with gradients of salinity, temperature and turbidity. Plumes or other forms of distribution of domestic, industrial or thermal effluents are examples of man-made gradients of environmental stresses. In both cases single or radially drawn transects through the centre of gradient fields make the most satisfactory grid for sampling stations. Along transects the stations are located in the subareas which are under the strongest influence of stress factors, and in transition zones at short intervals. However, with increasing strength of stress factors, i.e. with increasing distance from their sources, the intervals between stations can in most cases be much longer.

(3) Size and number of samples per station

Quantitative sampling in general, and particularly as needed for the assessment of pollution-induced modifications of benthic communities, is supposed to provide accurate and representative information on both species composition and abundance of each species in the community. Because of the great variability and unpredictable distributions of species and their populations, the theory and methods for quantitative sampling are generally far from perfect. When considering the number of samples to be taken, the only general rule is again "the more the better". As to the size of samples, it would however be wrong to take as large a surface of samples as possible because then the surface of the samples might be larger than the aggregated clumps of organisms, which obviously leads into statistically incorrect designs. Based on relevant references (Boudouresque, 1974; Gray, 1971; Holme and McIntyre, 1971; Longhurst, 1959), and on practical experience, the following suggestions are given for routine infrastation sampling design:

- for macrobenthic sampling on soft bottoms at each station a surface of 0.3 to 0.5 m^2 should be sampled as an integral of 3 to 5 grab samples of 0.1 m^2 each, taken at random within the area of a station;

- for meiobenthos at each station at least 5 randomly distributed cores 4 to 8 cm in diameter seem to be adequate;

- on hard bottoms or other habitats where manual sampling is possible or required, 3 to 4 squares or circles should be sampled, each with the minimum area of 400 cm^2.

Since these rules provide only a rough approximation to real needs in specific conditions of a given area to be investigated, it is most advisable to check the adequateness of the number of samples decided on before the start of the actual sampling programme. For this purpose at selected, representative stations a number of samples (3 to 10 grabs, corers or squares) should be taken and processed at a specific level as for regular samples. The number of species counted by individual samples, or better, the calculated similarity indices for pair-samples, are then plotted against the increasing number of samples or their increasing cumulative surfaces. From the curve obtained (rectangular hyperbola) at the transition from gradient slope to the asymtote level the minimum number of samples or minimum area can be deduced. For construction of the curve, see Figure 24 in section 2.4.9, and details in Boudouresque (1974).

(4) Temporal sampling frequencies

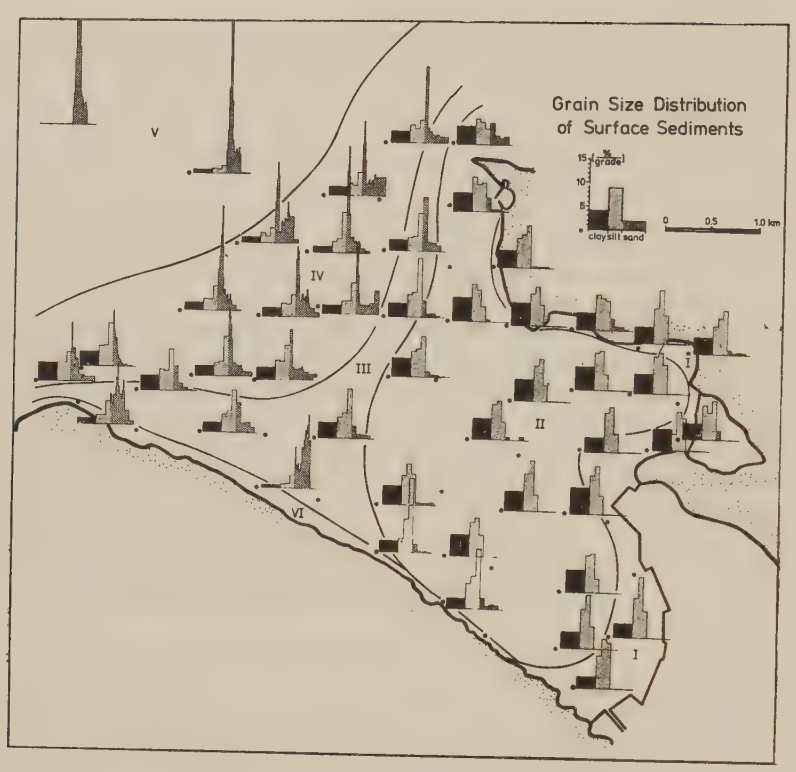
Although benthic communities are generally considered to be relatively stable in time as compared with pelagic biotic conditions, the seasonal dynamics of benthic populations must be considered when designing sampling programmes. Less evident (or less known) are the dynamics of "climax" communities at deeper soft and hard bottoms which occur mainly on account of seasonal recruitment peaks due to the settling periods of pelagic larvae or to particular grazing conditions. For such communities two sampling series (summer and winter) seem to be sufficient, provided that they are repeated over a number of years in order to record possible naturally occurring long-term fluctuations. In shallow littoral habitats where the vegetation is the dominating element the seasonal dynamics are very evident, and therefore the sampling frequency must be at least four times a year in accordance with the climatic seasons. However, it is suggested that some characteristic communities could be sampled with a monthly frequency.

The final sampling programme is designed, based on a compiled sampling map and a description of all tasks, using the above strategy and giving careful consideration to local conditions and needs. As an example, the sampling programme for a small but ecologically rather complex area of the Bay of Piran (North Adriatic) is shown in Figure 2 and briefly described as follows.

A. Information required before the final design of the sampling programme:

(i) Bathymetry of the whole soft-bottom area, geological composition and basic geomorphological forms of hard-bottom littoral shore and slopes;

(ii) Topographical distribution of the zones of characteristic types of sediments within the area and the data on their ecologically important parameters (description, granulometry, redox conditions, C, P, Ca, Fe and Mn content, semiquantitative description of biogenic artifacts, etc.);



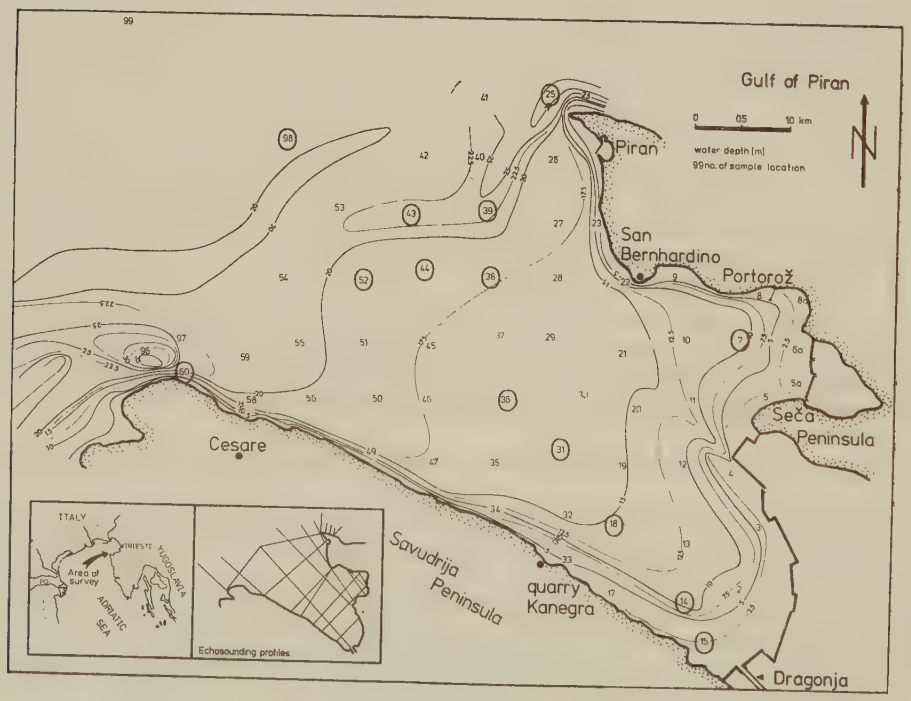


Figure 2. An example of stations grid for benthic sampling programme, including specific investigations at reference stations (circled)
(From Ranke, 1976, with kind permission of Senckenbergische Naturforschende Gesellschaft, Frankfurt. © 1976)

(iii) Oceanographic and pelagic bioproductivity data for typical annual seasons as obtained from measurements taken monthly over a period of two years:

- characteristic water masses, their spatial distribution and the basic features of their movements within the area;
- oxygen conditions generally, and particularly in near-bottom layers;
- pelagic trophic conditions of typical water masses generally, and particularly in the subarea of river discharges and of the submarine sewage outfall: inorganic nutrients P, N, Si, organic carbon and nitrogen, total and organic seston, phytoplankton populations and biomass, zooplankton biomass and typology, and total heterotrophic bacteria;
- light conditions in characteristic water masses generally, and particularly their gradients along selected underwater sampling transects on hard bottoms.

(iv) Qualitative information on the distribution of the six characteristic patches of benthic assemblages on the soft bottoms of the area, as obtained by dredging and diving observations (offshore detritic marl, central mud plain, inshore limestone and flysh detritic, Cymodocea seagrass prairie and estuarine stress subarea);

(v) Qualitative information (obtained from diving observations) on the geomorphology of representative sections of the hard-bottom slopes suitable for quantitative sampling along transects, and on the basic composition and zonation of relevant communities;

(vi) Information about quantities, composition and spatial distribution of pollutants discharged by river and submarine outfall, obtained from public services and previous investigations using pollution tracers (distribution of MBAS and faecal coliforms).

B. Benthic sampling programme:

(i) Soft bottom communities

The station grid comprises 36 basic stations and uniformly covers almost the whole area, except the stations for the patch of the seagrass community which are determined during sampling, and those in gradient subareas of the estuary and the submarine outfall where they are located along the local transects. In the transition zones between estuarine-central and central offshore subareas, the basic stations are complemented with additional ones. The total number of stations is 60. Stations deeper than 2 m are sampled by van Veen grab of 0.1-m^2 sampling surface, and shallower stations by divers using rectangular corers of 0.05-m^2 sampling surface. At each station 3 grab or rectangular corer samples are taken at random twice a year, in August and March. From each sample a 100-ml subsample of non-processed sediment is taken and preserved for possible future use; the rest is sieved immediately on board and preserved.

Fourteen stations (see Figure 2) are sampled for additional purposes. Therefore, at each station 6 tube corers (4-cm diameter) are taken at random, 3 are preserved for meiobenthos, 1 is dried for sedimentological and geochemical analyses, and 2 are deep-frozen for the detection of toxic elements and pesticides.

(ii) Hard-bottom communities

The following sampling transects from supralittoral to the deepest hard substrate are chosen:

- exposed limestone slope;
- exposed flysh slope;
- sheltered limestone slope without estuarine influence;
- sheltered limestone slope under estuarine stress;
- shallow, mainly artificial hard substrates influenced by the effects of urbanization.

Stations, i.e. horizons, are determined empirically in accordance with the pattern of the zonation of communities, as illustrated in Figure 3. In principle, there are 2 stations for supralittoral, 2 for mediolittoral, 3 for infralittoral, 2 to 8 for lower infralittoral (depending on the maximum depth of hard substrate) and 3 for upper circalittoral (existing only at exposed outer slopes). At each station 3 squares of 400 cm^2 surface are sampled manually from shore and by divers four times a year (May, August, November, March). Before sampling a larger area at each station (1 to 3 m^2) is observed, scattered megabenthic organisms are collected and the relative surface of the cover made by vegetation or sedentary fauna is recorded. All samples are processed alive, usually in the laboratory.

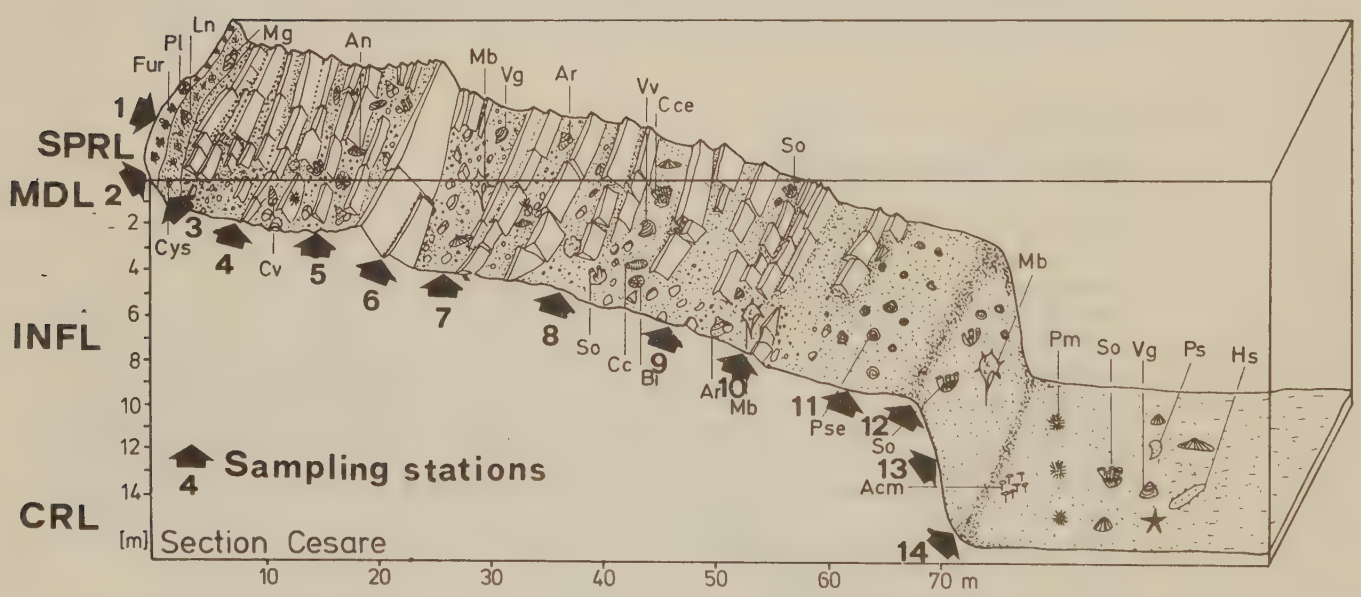


Figure 3. An example of sampling station distribution for hard-bottom benthos determined on the basis of previous knowledge on vertical zonation of communities
(From Ranke, 1976, with kind permission of Senckenbergische Naturforschende Gesellschaft, Frankfurt. © 1976)

SUMMARY

The basis for the design of an adequate sampling programme is the compiled environmental information on areas to be investigated, which should include:

- topography, bathymetry and sedimentology of the bottom and seashore presented in suitable maps;

- temporal and spatial distribution of typical water masses, their hydrographic and bio-productive conditions and dynamic transport, with particular emphasis on distribution of pollutants;

- sources, rates and composition of pollutants discharged in investigated areas.

In addition, the major types of benthic communities and their approximative distribution in investigated areas must be known before final sampling design. Floro-faunistic inventories and relevant reference collections, as complete as possible, should be prepared prior to quantitative investigations.

When all this information is available, the selected area can be topographically plotted into major subareas according to environment and communities and sampling stations are designed as follows:

- For homogeneous benthic assemblages (most soft-bottom communities, seagrass prairies) a grid is composed of uniformly located stations with at least one station covering each of the preliminarily identified patches and more in transitory zones.

- For gradient benthic zones, such as shore littoral communities (vertical zonation), estuaries (thermohaline gradients) and directly polluted zones (pollution gradient), the sampling stations are determined along perpendicular and radial (from gradient centre) transects. Similarly, for each distinct assemblage along the transect, at least two stations are determined.

At each soft bottom sampling station, a minimum of 0.3 to 0.5 m^2 of surface should be sampled as an integral of 3 to 5 grab samples taken at random. On hard bottoms, or on other habitats where manual sampling from shore or by diving is possible or required, a minimum of 3-5 squares, each with 400 cm^2 surface, are sampled.

Since the above rules provide only a rough approximation, it is recommended that an adequate number of samples per station be checked by a species-area test (see 2.4.9).

Temporal sampling frequencies of at least twice a year (summer and winter) for less dynamic communities (aphytal soft-bottom communities) and monthly for communities dominated by plants (algae, sea grasses) are recommended.

2.4 Sampling and Processing Methods

2.4.1 Ships and shipboard equipment used for sampling

The most suitable vessels for use by divers sampling on shore submarine slopes are medium-size inflatable boats, with 10 to 30 hp outboard motors. They can be used in very shallow waters and their safety and high speed ensures fast transfer of live samples to a laboratory or a mother ship for preliminary sorting. The boats should of course be properly fitted for safe diving operations. The equipment should include sampling gear as described below, and basic aids for navigation by landmarks, i.e. charts and triangles or a navigation protractor, and a good hand-bearing compass with prisms for azimuth determinations of landmarks. (For instructions on this method of determing the position of

stations or transects, see a basic maritime navigation manual). The position of submarine sampling stations along a transect can also be determined with a good underwater manometric depth meter, but the simplest way to do this accurately is to place fixed marks (metal or plastic nails) at the sampling stations, with a fixed mark at the beginning of the transect on shore.

For preliminary quantitative investigations on soft bottoms, the sampling gear required is small-size dredges and beam trawls which can be easily handled from small boats (7 to 10 m in length) with 20 to 30 hp diesel engines. In shallow waters (5 to 30 m) hauls can be made by three men manually; however, dredging vessels should be equipped with a boom and winch for up to 300-kg loads, especially if working in deeper waters. Large-size or anchor dredging can be handled only by larger vessels. The position of stations and transects can be determined by navigation using landmarks unless far offshore, when dredging operations must be carried out from radar-equipped vessels.

For use in quantitative investigations in coastal waters, small and medium-size fishing vessels, ideally stern trawlers, 15 to 25 m in length can be easily converted. The prerequisites are:

- cranes or booms and winches with 6 to 8-mm minimum cables, handling loads up to 600 kg;
- a source of running sea water at marine ambient temperature;
- sufficient free deck space (minimum 10 m^2) for handling samples and a safe storage room;
- good navigational facilities for position determination of stations, i.e. up-dated charts, navigation protractor, a good compensated ship's compass, and a pelorus or sextant for measuring horizontal angles needed for precise navigation by landmarks; radar or radio navigation is needed for work far offshore;
- standard recording echo sounder.

2.4.2 Sampling on hard bottoms

2.4.2.1 Qualitative sampling

Qualitative sampling and observations of communities are most successfully performed by diving; of course only the ecologists in the research team can do this job and therefore they must be fit, adequately trained and equipped. Scrapers, a geologist's hammer and collecting bags (made of fine-mesh netting, not plastic) are the only tools needed. Very useful information can be obtained by means of underwater photography or TV-magnetoscopic records of observed communities and habitats. Underwater notes and drawings can be made on aluminium or plastic plate with an ordinary pencil. Because of known technical and physiological problems a resonable limit for normal SCUBA divers should be set at 40 to 50 m; if deeper hard bottoms have to be investigated, professional divers or the crew of a submarine vessel should be employed.

If deep hard bottoms are not too steep, or if they are enclaved within plain soft bottom, a number of heavy duty dredges can provide satisfactory qualitative samples.

For this type of work the Naturalist's rectangular dredge, heavy type (45.7-cm opening with 12-mm mesh nylon bag) is recommended (see Figure 4). It can be home-made or supplied by the Marine Biological Laboratory in Plymouth, U.K. Due to rocky or encrusted irregularities of hard bottoms, the cables and other gear connected to the dredge must be strong enough to hold forces up to 1000 kg. The dredge is equipped with a "weak link" (shown on Figure 4), consisting of several turns of twine (for heavy-duty three turns of 8-mm manila rope), which breaks if the dredge is anchored or stuck between rocks, allowing the arms to open out and free it. The dredges are towed at a minimum speed (1.5 knots) or just by drifting. For a normal operation, it is suggested that warp at least equal to twice the depth of dredged hard bottom be paid out; towing time should be at least 15 minutes.

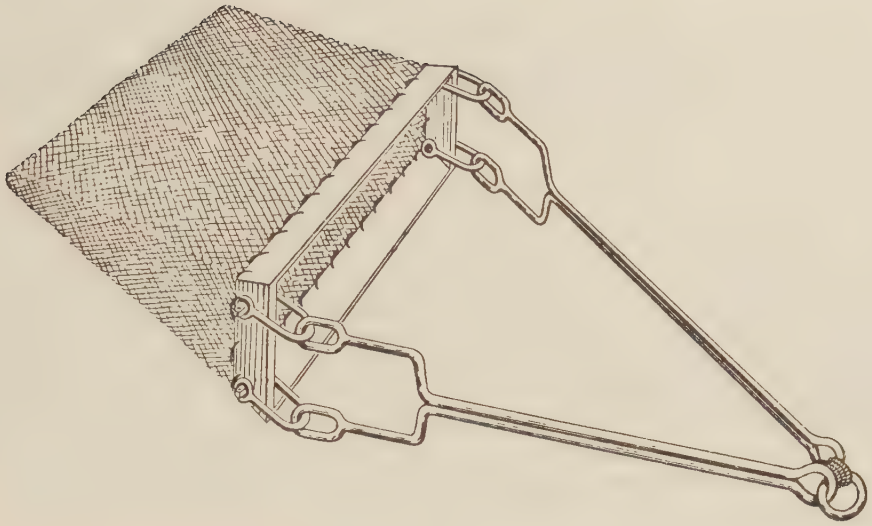


Figure 4. Naturalist's dredge. Note the position of a "weak link"
(From Holme and McIntyre, 1971, with kind permission of the International Biological Programme, London. © 1971)

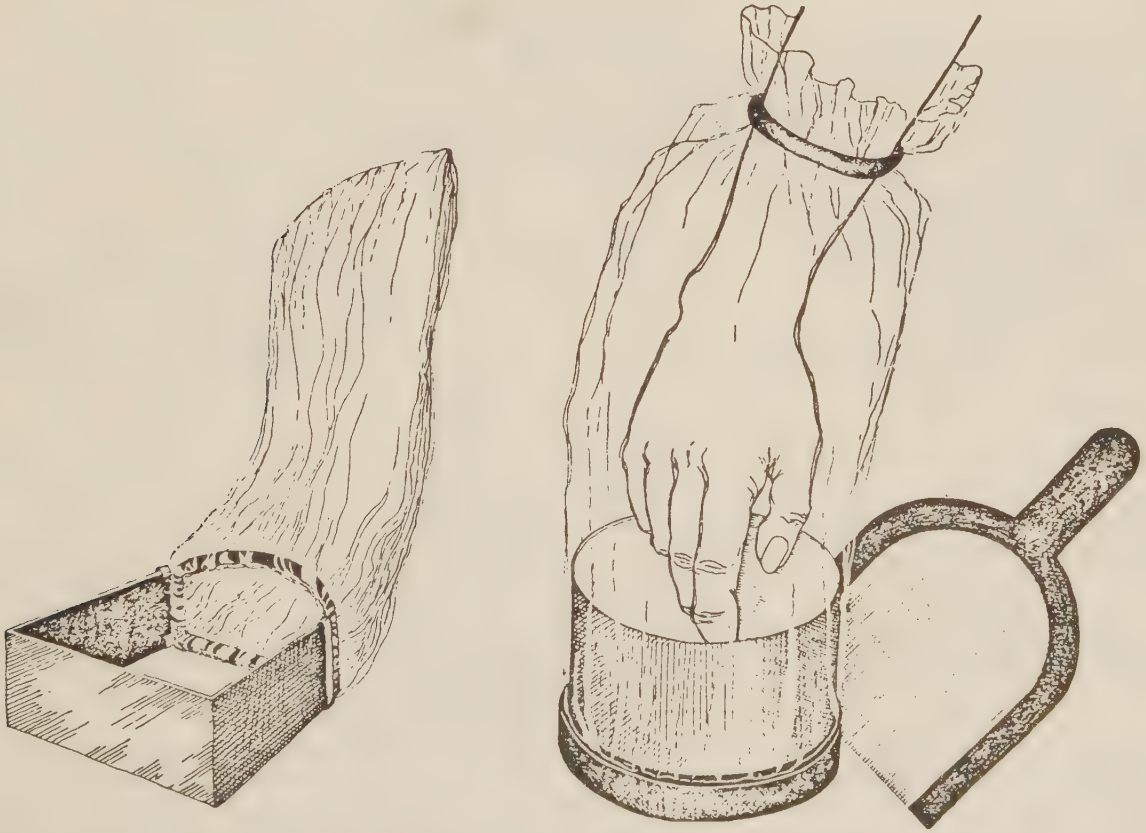


Figure 5. Some accessories for manual quantitative sampling on hard substrates
(From Kautsky, 1974, and Jansson, 1974, with kind permission of the University of Stockholm. © 1974).

2.4.2.2 Quantitative sampling

Quantitative sampling on hard bottoms can only be performed manually. This can easily be done from shore at low tide for supralittoral, mediolittoral and upper infralittoral communities; in deeper zones, however, the sampling can be done only by divers.

The recommended method (see 2.3.3) is the sampling of 400 cm^2 surface units of a substrate from where the total complex of biota is scraped off as carefully as possible into a collecting bag (0.5-mm mesh, or 0.1 mm if meiofauna is required too). Before square samples are taken the cover made by dominant, usually algal, agglomerations is estimated and recorded in arbitrary units of 0, 5, 10, 25, 50, 75 and 100 percent of the covered surface. For collecting samples from dense and easily removable assemblages a flexible aluminium frame (inside dimensions 20 x 20 cm) with a collecting bag, fastened to the lower margin of the frame, is fixed onto substrate with some nails and biota carefully and systematically scraped into the collecting bag. If the assemblages are very fragile or easily disturbed, the whole sampled surface must be tightly connected to the sampling bag and the scraping done from inside, as shown in Figure 5. When the smallest vagile elements of meiofauna have to be caught quantitatively too, there are more sophisticated hydraulic or suction samplers for hard-bottom substrates (see Figure 6); for further details see Hiscock and Hoare (1973).

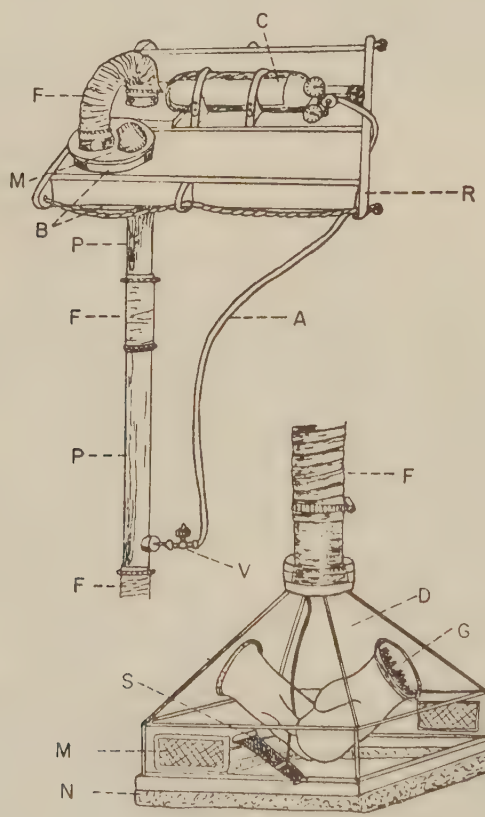


Figure 6. Suction sampler for hard bottoms: **A** - air hose; **B** - plastic bucket - with open bottom covered with fine steel mesh; **C** - air cylinder; **D** - perspex funnel; **F** - flexible pipe; **G** - latex glove; **M** - stainless steel mesh; **N** - foam plastic; **P** - rigid plastic pipe; **R** - raft; **S** - scraper; **V** - air valve
(From the Finnish IBP-PM Group, 1969, with kind permission of Akademie Verlag, Berlin. © 1969).

Besides the described square samples described, larger areas, optimally 3 squares, each of 1 m^2, should be sampled manually for megabenthic organisms, large species of algae, sponges, anthozoans, molluscs, decapods, aggregated bryozoans, echinoderms and ascidians), which have a scattered distribution and therefore their presence in the samples would be incidental.

2.4.3 Sampling on soft bottoms

2.4.3.1 Qualitative sampling

For shallow intertidal and upper infralittoral zones the most convenient way to collect infauna and most epifauna is by simply digging sedimentary substrate. A square sheet metal frame, for instance 0.25 m^2 surface, is driven into the substrate and all sediment excavated with a spade or a large scoop to a depth of 20 to 30 cm and collected in buckets or sieved on the spot on 1 to 2-mm screens. Scattered epibenthic and demersal nektonic organisms can be collected simply by systematic screening of a larger epibenthic surface with a scoop net (basal opening 50 cm, netting bag of 0.5-cm mesh), as shown in Figure 7.

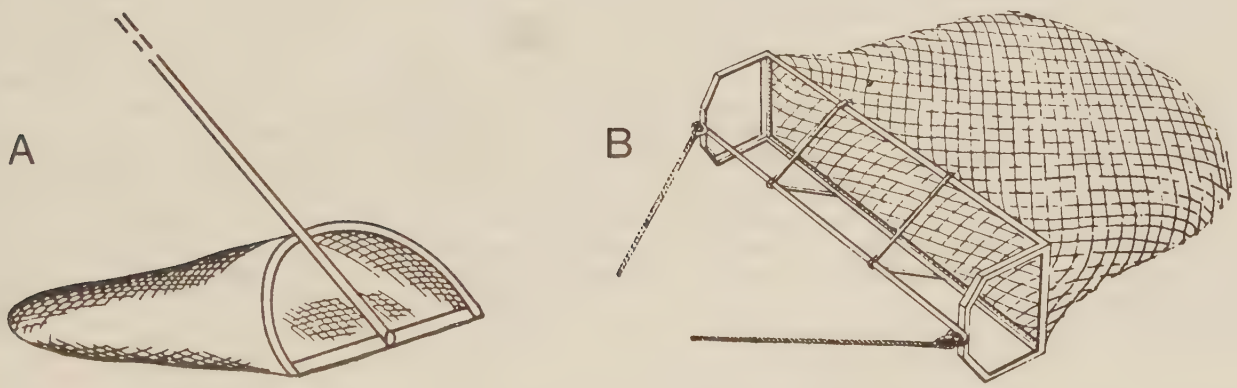


Figure 7. Gear for qualitative sampling on soft bottoms: **A** - push scoop net; **B** - Kiel dredge (From Schlieper, 1972, with kind permission of Sidgwick and Jackson Ltd., London. © 1972)

In deeper waters, besides diving or TV observations, various dredges are used. However, it is advisable also to use grab sampling for preliminary investigations in order to get some quantitative estimates before the final sampling programme design.

Dredging for epibenthic megafauna is a relatively easy task. Many types of dredges and beam trawls are suitable for this purpose, and can be made quickly by a local fisherman, such as the simple beam trawl with 1.5-cm mesh bag (Fig. 8), or epibenthic dredges, such as the Kiel dredge (Fig. 7) (suitable dimensions of the opening are 110 x 30 cm). Epibenthic dredging is carried out in the same way as described for the rock dredge (see 2.4.2), but it is advisable to pay out warp equal to three times the depth of the dredged bottoms. For towing a 12-mm synthetic rope can be used; steel cables are less practical unless working on larger vessels.

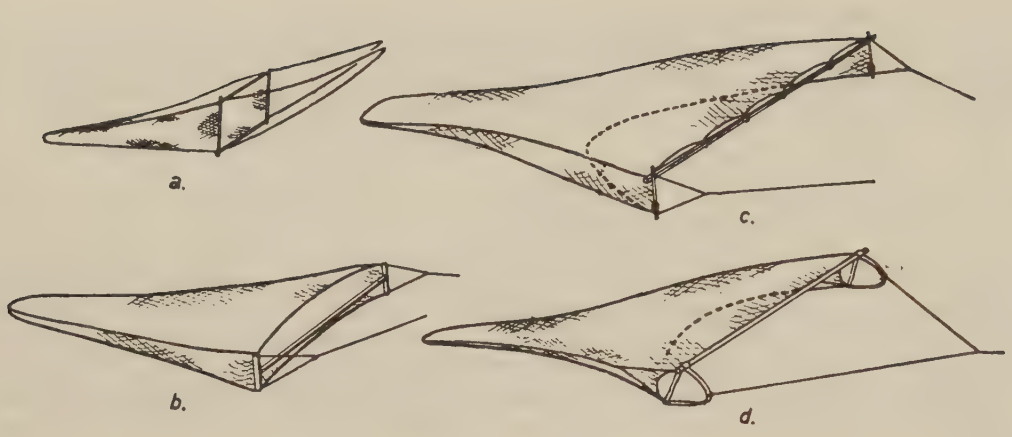


Figure 8. Beam trawls for qualitative sampling
(From von Brandt, 1981, with kind permission of the author. © 1981)

Except on very soft muddy bottoms, epibenthic dredges are not generally used for sampling burrowing infauna, not even while performing preliminary investigations. Therefore, an anchor dredge is suggested such as the Forster's type (see Figure 9), which can be supplied by the Marine Biological Association, Plymouth, U.K. According to Holme and McIntyre (1971) this dredge is one of the most useful instruments available for sampling sands and similar firmly packed deposits. It digs deeply at one place on the sea bed, and is not towed along as are other dredges. The anchor dredge is shot and hauled in the same manner as a ship's anchor; it is most conveniently shot over the side, with the ship going astern and, provided there is sufficient way on the ship for the net to stream out during lowering, it can usually be landed on the bottom right way up. As the ship drifts slowly astern, warp equal to about five times the depth is gradually paid out. The inboard end is then made fast, and the strain exerted as the ship is brought to a standstill drives the dredge into the sand to a depth of 25 cm. The ship then steams slowly ahead as the warp is heaved in so that the dredge is broken out from the bottom when the warp is almost vertical. Another type of anchor dredge is the one described by Sanders et al. (1965). See Figure 10.

2.4.3.2 Quantitative sampling

(a) Shallow macrobenthos - Since appropriate grabs must be operated from large vessels they cannot be used in very shallow waters, less than 2.5 to 3 m deep. Such bottoms, including seagrass prairies, must be sampled manually - from land for intertidal and by diving for infralittoral zones. The simplest method would be excavation of a substrate from a frame as described for hard bottoms, provided that sediments are firmly packed and epibenthic or demersal nektonic elements in the sampled community are prevented from escaping; otherwise this method cannot be recommended as a general approach.

There are a number of shallow benthic samplers developed (Kangas, 1972) which can be satisfactorily used for quantitative sampling; the following are recommended:

- mechanical self-closing sampler (O'Gower and Wacasey, 1967), as shown in Figure 11, which collects samples of a 15 x 15-cm surface and can penetrate up to a depth of 20 cm. Its only disadvantage is that speedier vagile organisms escape of some epibenthic elements may be flushed out; however, this can be prevented by inserting a netting bag above the upper opening of the sampler. The samples are collected by pushing the sampler vertically into the substrate until part of the hinged side is embedded to the level of the opposite side of the sampler. The hinge locking pin is then withdrawn and the sampler pushed further down into the substrate until the hinged side swings underneath, because of the convergent angles of the sides of the sampler, to become the closed base holding the sample.

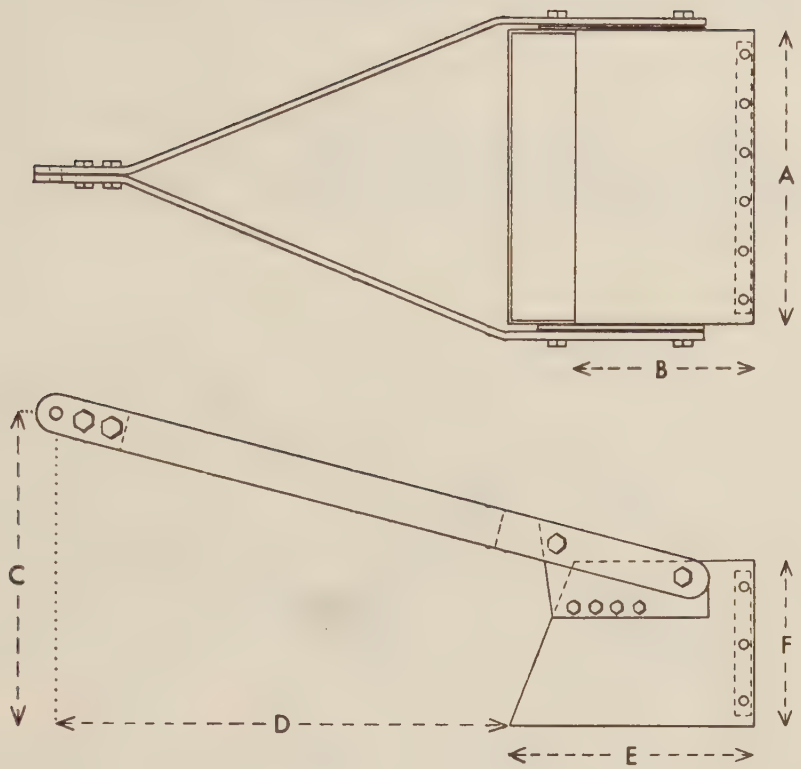


Figure 9. Anchor dredge type Forster, Plymouth
(From Holme and McIntyre, 1971, with kind permission of the International Biological Programme, London. © 1971)

The dredge is made up of mild steel in two sizes:

	Medium	Small
Wishbone towing arms	64 x 12.5 mm	51 x 6.5 mm
Sheet for scoop	5	3
Dimensions		
A	457	350
B	279	203
C	490	384
D	724	457
E	381	279
F	260	184

The net is attached by eyelets clamped between strips of metal bolted around the four sides of the scoop. These have bolts of about 10 mm diameter every 50 mm.

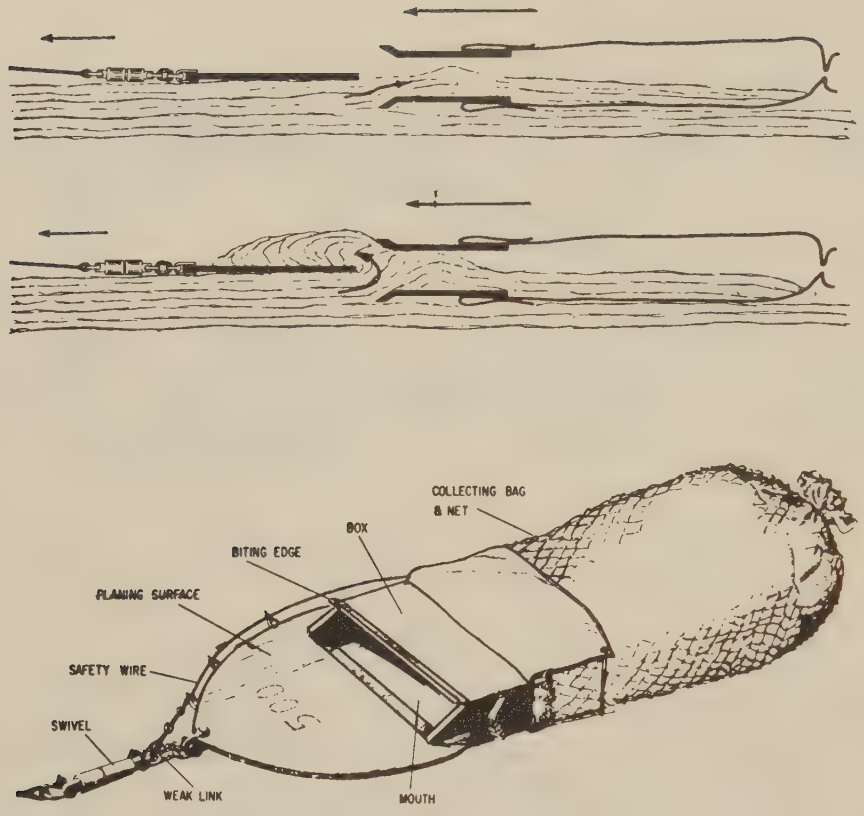


Figure 10. Anchor dredge Sanders type
(From Sanders et al., 1965, with kind permission of Pergamon Press, Oxford. © 1965)

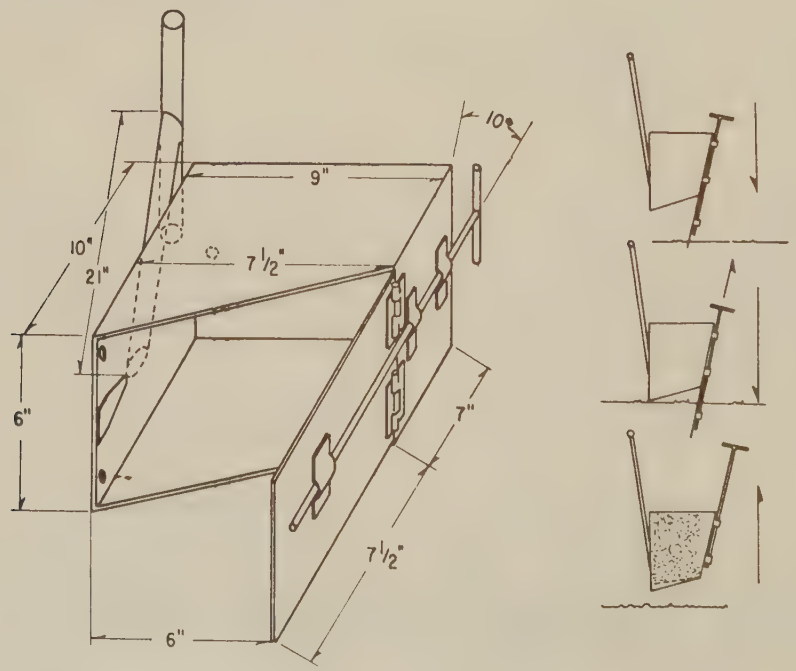


Figure 11. Mechanical self-closing sampler for shallow bottoms
(From O'Gower and Wacasey, 1967, with kind permission of University of Miami, Miami, Fla. © 1967)

- The Štirn and Vrišer coring sampler (Fig. 12), made of strong acrylic tube, 18 cm in diameter, which was developed in order to sample shallow benthos together with the water layer above and its demersal nektonic organisms, and particularly for seagrass prairies and lagoon communities. The sampler covers a surface of 270 cm^2, penetrates up to 25 cm into the substrate and also collects a 30 cm high column of water above sediments. The sampler, with the top open, is placed quickly in a vertical position on the bottom and pushed a few centimetres into the substrate; then the stopper is loosely inserted and the sampler pushed further into the substrate until the water discharge valve reaches the water. Before the sampler is pulled out, the stopper is inserted tightly and both base arms turned 180° in order to hold the sample within the sampler. When the sampler is landed, the water column is discharged into a conical 1-mm mesh net, obtaining nektonic and some vagile epibenthic organisms, and then the benthic fraction is sieved and preserved.

(b) Sublittoral and bathyal macrobenthos - Sampling macrobenthos from shelf soft bottoms usually presents the main focal task in any pollution-oriented benthic investigations. There are many methods and various types of sampling gear; for a complete review, see Holme and McIntyre (1971). Although diver-operated suction samplers such as those recommended by Barnett and Hardy (1967) or Massé (1970) are excellent for use in shallower shelf zones and quantitative anchor dredges such as Sanders et al. (1965) suggest are most probably more reliable for use in bathyal zones than many sophisticated grabs, the routine sampling for the time being will still depend upon relatively simple grab methods. Therefore, and also for reasons of economy, details are given mainly on the 0.1-m^2 van Veen grab which can be recommended for routine sampling (Fig. 13). For construction details (if home-made) see Dybern et al. (1976). The grab can also be purchased from suppliers (for instance, Benthos Inc., N. Falmouth, U.S.A.; Hydrobios, Kiel, Federal Republic of Germany; Laboratoire Océanographique, Charlottenlund, Denmark).

The grab has a sampling area of 0.10 m^2, and weighs 25 to 35 kg empty. It should be modified from the original van Veen grab as follows:

- The release mechanisms first introduced for use with the van Veen grab by Ursin (1954) should be used. When premature closing occurs due to weather conditions, the modification described by Lassig (1956) can be used.

- In order to reduce the shock wave caused by the grab, the windows on the upper side should cover as lage an area as possible (minimum 60 percent of the upper surface of the grab). The windows should be covered with metal gauze of 0.5 x 0.5-mm mesh size.

- Means should be provided for attaching an extra 20 kg of lead weights. This is perhaps best done by fastening four equal pieces of lead on to the upper edges of the jaws.

The following precautions should be observed when using the grab:

- The setting down and closing of the grab should be done as gently as possible; this will reduce the shock wave and the risk of loss of sediment by lifting the grab before complete closure.

- The wire angle must be kept as small as possible, to guarantee that the grab is set down and lifted up as vertically as possible.

- If, as often happens on sandy bottoms, less than 4 l of sediment is collected, the sample may be used, but the low sample volume should be stressed when results are given.

- Each investigator should carefully check the exact sampling area of this particular grab, and calculate per m^2.

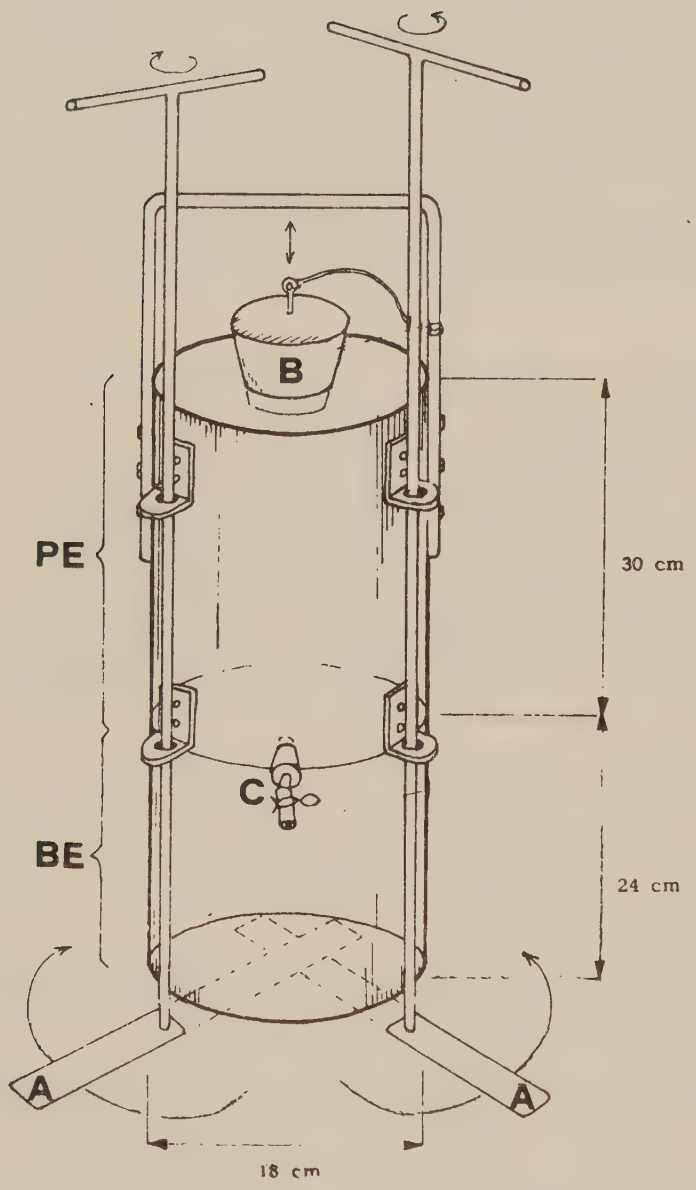


Figure 12. Combined benthic-pelagic sampler for shallow water: **A** - sediment-holding arms; **B** - stopper; **C** - pelagic phase discharge valve; **PE** - pelagic phase; **BE** - benthic phase

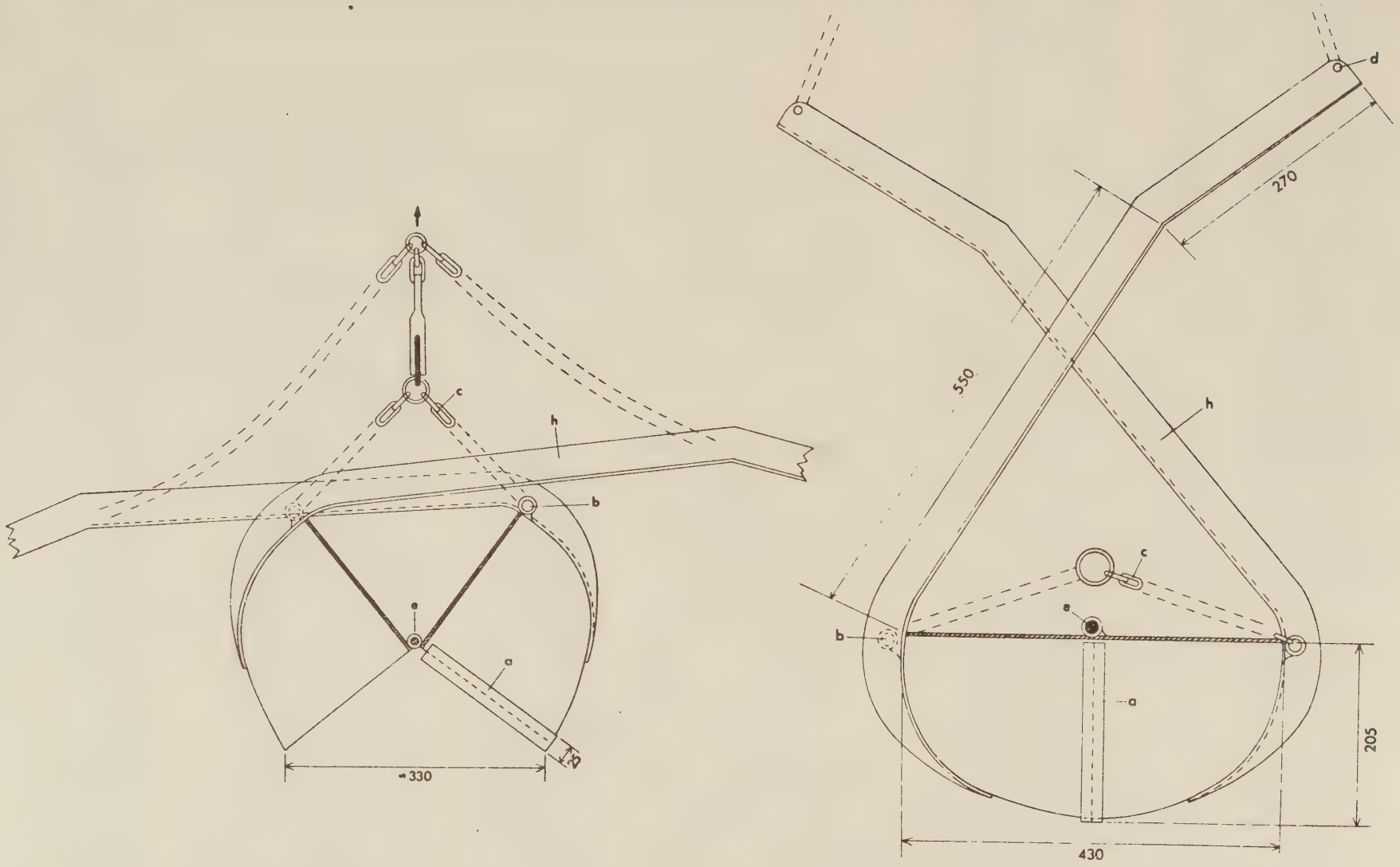


Figure 13. van Veen grab (0.1 m^2)
Left: Long side view, open mode. Dimensions in mm. Jaws of galvanized iron plates, thickness 4.5 mm. **a** - metal band that ensures tight fit of right jaw over left jaw, thickness 4.5 mm; **b** - metal ring for attachment of chains; **c** -lower chains, length 260 mm, used for releasing mechanism; **e** - axis, diameter 15 mm; **h** - arm, of L-shaped galvanized girder, 50 x 50 x 5 mm. Gap distance can be adjusted by bending one of the arms to give a 0.10 m^2 sampling area. **Right:** Long side view, closed mode. Dimensions in mm. **d** - hole for attachment of upper chains (length 900 mm) by means of shackles
(From Dybern et al., 1976, with kind permission of the University of Stockholm. © 1976).

Benthic samples also recommended are the 0.1-m^2 "orange-peel" grab, as modified by Briba and Reys (1966), and the 0.1-m^2 Smith-McIntyre grab (Holme and McIntyre, 1971). The latter, which is actually a spring-loaded version of the van Veen grab, is perhaps the optimal gear available at present. It is advisable to purchase such samplers from commercial suppliers since they are not easily home-made.

As to the handling of the grab at sea, the prerequisite ship equipment has been mentioned above (see 2.4.1), but the following advice given in Holme and McIntyre (1971) is of particular importance for the success of grab sampling:

- It is important not to use too heavy a wire for the purpose; most grabs are not hydrodynamically shaped and sink rather slowly, so that if too heavy a wire is used it may form a loop below the grab, possibly causing kinking. For similar reasons the grab should be lowered steadily with gentle braking on the winch.
- As soon as the bottom is reached, the brake should be applied and hauling commenced immediately. Any delay on the bottom will increase the wire angle if the ship if drifting, causing the instrument to be pulled out obliquely, making it work less efficiently.
- It is important to haul very slowly until the sampler has left the bottom.

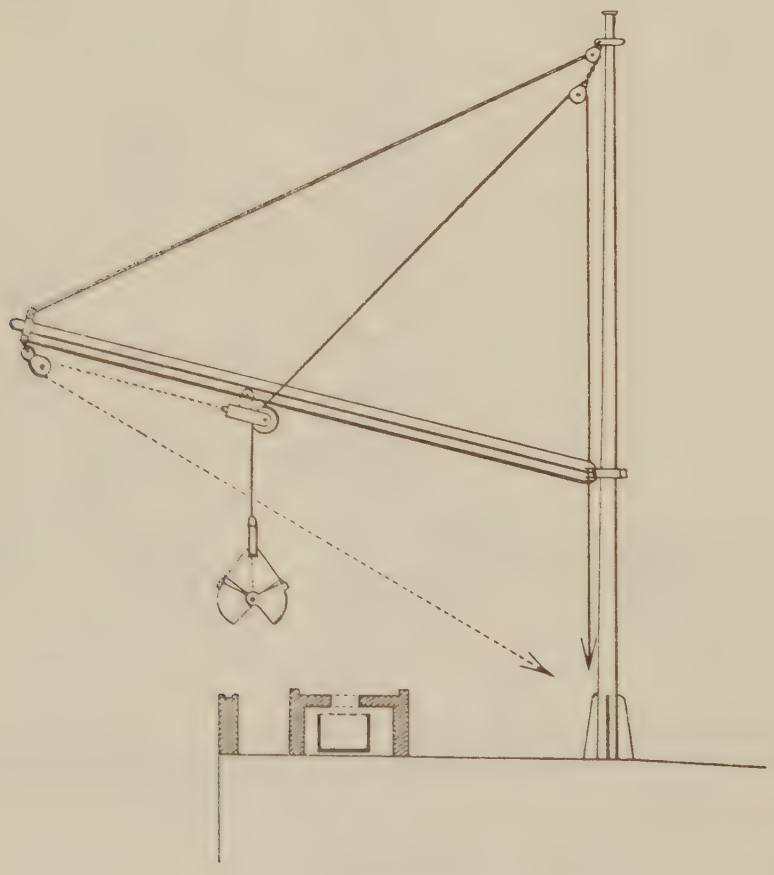


Figure 14. Deck arrangement and the system for outhauling grab using jockey pulley
(Fom Holme and McIntyre, 1971, with kind permission of the International Biological Programme, London. © 1971).

The most convenient wire for the 0.1-m^2 van Veen grab is of 6 mm diameter, provided it is of good quality and not damaged. Between the grab and the wire a strong swivel must be inserted. The most efficient deck arrangement for grab operations is shown in Figure 14.

(c) Meiobenthos - Considering spatial distributions and consequent needs for adapting different sampling techniques, there are three basic types of meiobenthos (see also 2.2.2 above):

- Meiofauna as a part of dense benthic assemblages on hard bottoms. For this type no specific sampling has been developed, except the very successful and quantitative scraping of total biota from the mineral substrates with the help of a suction or hydraulic sampler as described in paragraph 2.4.2.2.

- Interstitial meiobenthos of sorted sandy substrates, which requires long cores since the meiofauna can penetrate deeply into the sediments. The most adequate samples are obtained by means of a cylindrical tube (transparent synthetic material, 3 to 4 cm. in diameter, sharpened at the lower end), open at both ends, which is pushed very slowly into the sediment for at least 20 cm. The upper end of the tube is then stoppered, the sediment around the tube excavated so that the tube can be stoppered at the lower end also, and only then can the sample be collected.

  Treatment of meiobenthic samples in the field or on smaller ships is usually not possible. Therefore, the sample as a whole or in portions is transferred from the corer into appropriate jars and the inside of the corer washed in sea water which is added to the samples in jars. Samples to be treated in the laboratory alive are stored in a cool place (maximum temperature 10°C); otherwise they are immediately preserved. Often the vertical distribution of meiofauna within the sediment column is studied. For such purposes the sectioning of the core must be done immediately upon collection to avoid errors occurring because of subsequent redistribution of organisms within the sample. This can usually be done by sliding the core out of the lower end of the corer for the required section (2.5 or 10 cm) which is then sliced off into a jar. To help the sliding, gentle pressure on the side of the upper rubber stopper allows air into the top of the corer.

  On deep bottoms, or if divers are not available, similar cores are taken by a suitable core sampler (see 2.4.10 below). Samples for meiofauna obtained by coring of grab samples can be considered only as semiquantitative, except if only surface layers of very compact substrates are considered or special grabs such as the Reineck sampler were used. For extensive information see Dybern et al. (1976); Holme and McIntyre (1971), and Hulings and Gray (1971).

- Mud meiobenthos, which, in contrast to the interstitial fauna, is concentrated in the top surface layers of substrates since there is no space in compact, anaerobic deeper layers, except for some burrowing organisms - mainly nematodes and meiobenthic annelids. Therefore, there is no need for deep sampling, but great care should be taken so that the uppermost layer is sampled with as little disturbance as possible. Generally the sampling is carried out as described for interstitial fauna. However, the cores should not be longer than 10 cm, and in the sectioning at least the upper 2-cm layer should be treated separately from the rest of the core. For the mud meiobenthic sampling and sectioning, the most efficient method seems to be the one described by Ankar and Jansson (1973), shown in Figure 15.

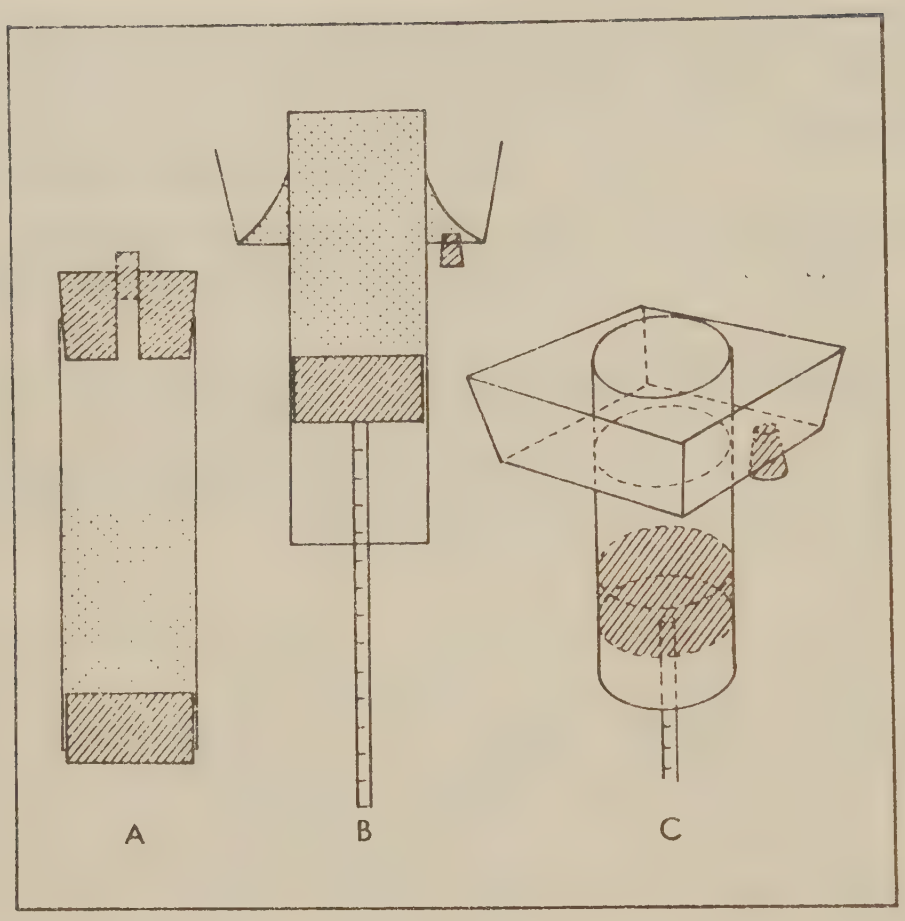


Figure 15. Schematic drawing of simple acrylic glass corer for SCUBA sampling of meio- and/or macrofauna. A - plexiglass tube with sediment core and rubber stopper in place. The upper stopper has a small hole for equalization of pressure as the lower stopper is pushed into place. This small hole is then plugged with a small rubber bung. B - acrylic glass tube with piston on cm-graded stick, for extrusion of core in cm-layers. C - side view of B.
(From Ankar and Jansson, 1973, with kind permission of Springer Verlag, Heidelberg, and the authors. © 1973).

SUMMARY

The following methods for benthic sampling are recommended:

(a) Qualitative or semi-quantitative sampling is done manually on shore or by divers on hard bottoms, possibly accompanied by underwater photography or TV-magnetoscope, and by using dredging gear such as the Naturalist's on hard bottoms or the Kiel dredge on soft bottoms.

(b) Quantitative sampling on hard bottoms can be properly carried out only by divers, except in the intertidal zone; total biota, from a minimum 400-cm^2 surface, is scraped off and put into a collecting bag in a sizeable quantity; prior to that, the relative cover made by agglomerated or encrusting species is determined.

(c) Quantitative sampling on very shallow soft bottoms and in seagrass prairies is done manually from the shore or by divers by large-size cover samplers such as the O' Gower and Wacasey self-closing sampler or Štirn and Vrišer coring sampler.

(d) Deep soft-bottom quantitative sampling is carried out with 0.1-m^2 grabs such as the van Veen, Smith-McIntyre or Briba and Reys' modified "orange-peel" sampler. Alternatively, samples can be obtained by diver-operated suction samplers such as Barnett and Hardy's or Masse's.

(e) Meiobenthic samples from soft bottoms are obtained by manual or mechanical coring into substrates with acrylic glass tubes 3 to 4 cm in diameter; meiobenthic samples from hard bottoms are obtained as described in 2.4.3.2(c) above.

2.4.4 Treatment of samples

2.4.4.1 Hard bottom samples

As already mentioned, the best results can be obtained if samples are treated alive. Although the sorting of living organisms is easier, specific preservation methods can be selected. The first step in the treatment of the individual sample is careful washing with plenty of cool sea water on a 0.5-mm sieve in order to get rid of sediment particles, small detritus and debris (washing not to be done in the case of meiofauna). Then the larger solitary specimens or non-encrusting colonies of macroalgae, sponges, cnidarians, molluscs, decapods, bryozoans and ascidians, are quickly picked out from the sieve to be preserved separately; but they must first be carefully examined for any adhered or interstitial smaller organisms, in which case they are returned to the bulk sample. The remaining sample is transferred from the sieve into a large container with cool sea water. Smaller portions of the sample are then taken out separately and placed in plastic or enamel trays (such as those used in photo laboratories) and manually sorted into major taxonomic groups and/or immmediately recognizable species. Most of the sorting can be done without a microscope, but a good binocular stereo-microscope, petri dishes and dissecting tools must be available for checking that no macrobenthic organisms have remained in the sorted subsamples, and also for morphological observations needed to distinguish some of the more problematic taxonomic groups, such as cnidarians and bryozoans. All groups or species are sorted separately into suitable jars with cool sea water and only after the whole sample has been sorted out are these taxonomic subsamples preserved (see 2.4.5 below). If further identification is needed, however, all specimens of those species which are definitely identified at the first stage are ready for the biomass measurement. Until this procedure begins they can most conveniently be stored in a deep-freezer. It is generally recommended that biomass determinations are made on non-preserved fresh or frozen material, particularly for biomass-dominant species and specifically for macroalgae and for sponges, which lose quite a large proportion of organic matter by dissolution in preserving liquids. Therefore, it is advisable to preserve for further identification only very small specimens, or a fraction of them (in the case of sponges), while the rest is deep-frozen or directly used for biomass determination.

When it has not been possible to determine the relative cover in the field (see 2.3.3(B)(ii)), this must be done before the above procedure starts: the whole sample is distributed on the bottom of a large tray with sea water in approximately the same pattern as it was in nature, and then relative cover as made by dense sedentary populations is determined.

If the same samples are also to be used for the investigations of meiofauna, the original sample, not being sieved at all and without addition of sea water, except if it was previously filtered through a mesh of 20 to 40 µ, is quickly sorted for macrobenthic species. However, only those solitary and distinct species are sorted out which by their body structure do not offer interstitial space for meiofauna, such as dense algae covers, some sponges, cnidarians, polychaete colonies and bryozoans. The remaining sample, with the originally introduced sea water is then carefully separated and mixed up. A meiobenthic subsample, for instance, 1/4 of the total sample, is taken and treated by the Uhlig extraction method described in 2.4.4.3 below. When extraction is completed, the subsample can be sorted for remaining macrobenthic elements and incorporated into the sorted categories of the main part of the sample.

2.4.4.2 Soft bottom macrobenthic samples

Quantitative samples obtained by the recommended grab methods will consist of bulk sediment, including organisms which must be extracted as soon after the samples were taken as possible, i.e. on board the research vessel. In principle, this immediate extraction is done by sieving the whole sample on a 1-mm mesh sieve with the help of a sea water sprinkler in order to get rid of the bulk of sediments and detritus (Fig. 16). The remainder is then transferred into sample jars, preserved, labelled and separated later in the laboratory. It is, however, advisable to use series of mesh sizes, e.g. 0.5, 1.0 and 10 mm, during the sieving, for the following reasons:

- 0.5-mm sieve retains specimens of macrofauna which, due to their elongated form, may pass a 1.0-mm sieve, although they are usually longer;

- a 10-mm sieve separates coarse mineral or detritic particles and megabenthic species for the bulk of macrobenthic infauna usually obtained by a 1.0-mm sieve; dead material can be stored separately in dry conditions if needed for further studies, or just rejected, while megabenthic specimens are preserved in separate jars in order not to damage delicate infauna during transportation and storage of samples;

- separation and sorting of fractionated samples is easier and more convenient.

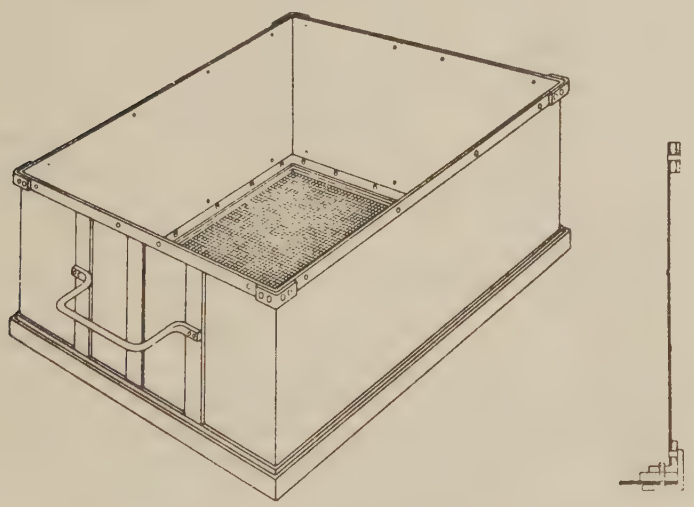


Figure 16. An example of a convenient sieve construction (From Edmondson and Winberg, 1971, with kind permission of the International Biological Programme, London. © 1971)

Screens are made from high quality stainless steel or bronze gauze at the bottom of stainless steel or plastic frames 15 to 25 cm high, depending on the sieving procedure to be applied. The free surface of the screens should be about 1 000 cm^2, or 30 x 30 cm; the outer surface must be reinforced, for instance, by a stainless steel cross. If using a series of screens it is convenient to construct frames in the form of drawers to be placed in a rack-like stand. Ideally, they should be made to fit completely into a large plastic or enamel tray so that all the screened material can be shaken down at once from the sieve into the tray.

The total sample or portions are transferred from the grab into the upper sieve and then the sieving is done by washing the material with gentle jets of sea water, shaking by hand and separating agglomerations. Fixed sprinkler-tubes or flexible heads, such as a shower nozzle, must be used for washing. For large sampling programmes, and if working on heavy seas, more robust systems for sieving operations are recommended, such as the Holme's hopper (Holme and McIntyre, 1971) shown in Figure 17. If no running sea water is available, the simplest sieving method is to transfer a portion of a sample into a sieve or tightly connected series of screens placed in a fairly large bucket with sea water, and to shake continuously until the sediments are washed out.

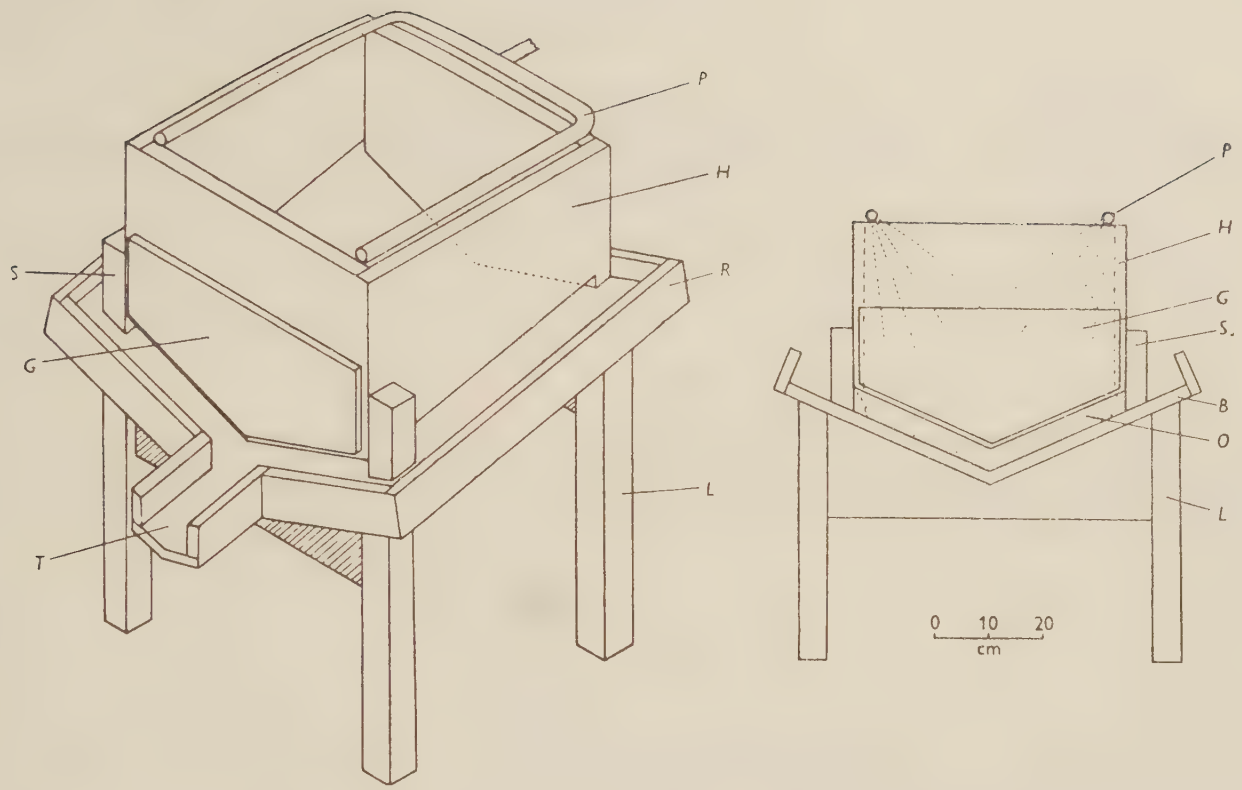


Figure 17. Holme's hopper for sieving benthic samples. **P** - pipes supplying jets along top of hopper; **H** - side-wall of hopper; **R** - retaining wall at side of base (**B**); **T** - spout; **G** - rising gate; **S** - short legs supporting hopper off base; **L** - legs; **O** - sediment seen through gap between hopper and base
(From Holme, 1959, with kind permission of Cambridge University Press, Cambridge. © 1959)

It should be stressed, however, that all procedures described above may damage more delicate organisms, particularly polychaetes. Therefore, Sanders' extraction methods (Sanders et al., 1965), is recommended for high-level sampling programmes (Fig. 18). The sample is washed by putting it in a large garbage can which has a spout near the top, much like a coffee pot. A large diameter water hose (e.g. 4 cm) is pushed down into the sediment, and a large volume of water running at a low velocity is pumped through the sediment. The resulting suspension of animals and fine-grained sediment pours out the spout and then through a 0.42-mm mesh screen. The animals are retained on the screen. Large animals are immediately picked out and preserved. At the end of the washing process there are three fractions: animals taken out, the fauna retained by the screen and a coarse fraction remaining in the can, consisting of coarser sediments (heavier organisms such as molluscs). The three samples are preserved separately. This method is time consuming but it is also extremely gentle, and in general the animals are well preserved and relatively undamaged.

Whichever sieving techniques were applied, the screened samples consist of a great variety of components: coarse sediment particles, skeletal artifacts of biota, detritus, urban and industrial waste items and living benthic organisms. The only way to extract and separate biota from the remainder is manual sorting (described in 2.4.4.1). Sorting of live samples is again recommended as an ideal approach. Unfortunately, during soft bottom sampling operations this is rarely feasible and therefore the samples must be separated in a preserved condition.

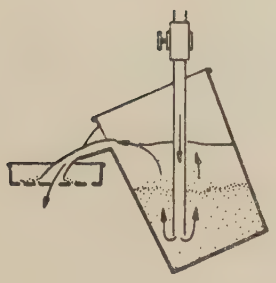


Figure 18. Overflow elutriation system
(From Sanders et al., 1965, with kind permission of Pergamon Press Ltd., Oxford. © 1965)

2.4.4.3 Meiobenthic samples

Extraction, separation and sorting of meiobenthic organisms, particularly if needed for reliable quantitative investigations, present a difficult task, and the most time-consuming part cannot be done with the naked eye; all operations, except the extraction, must be performed under a binocular dissecting microscope.

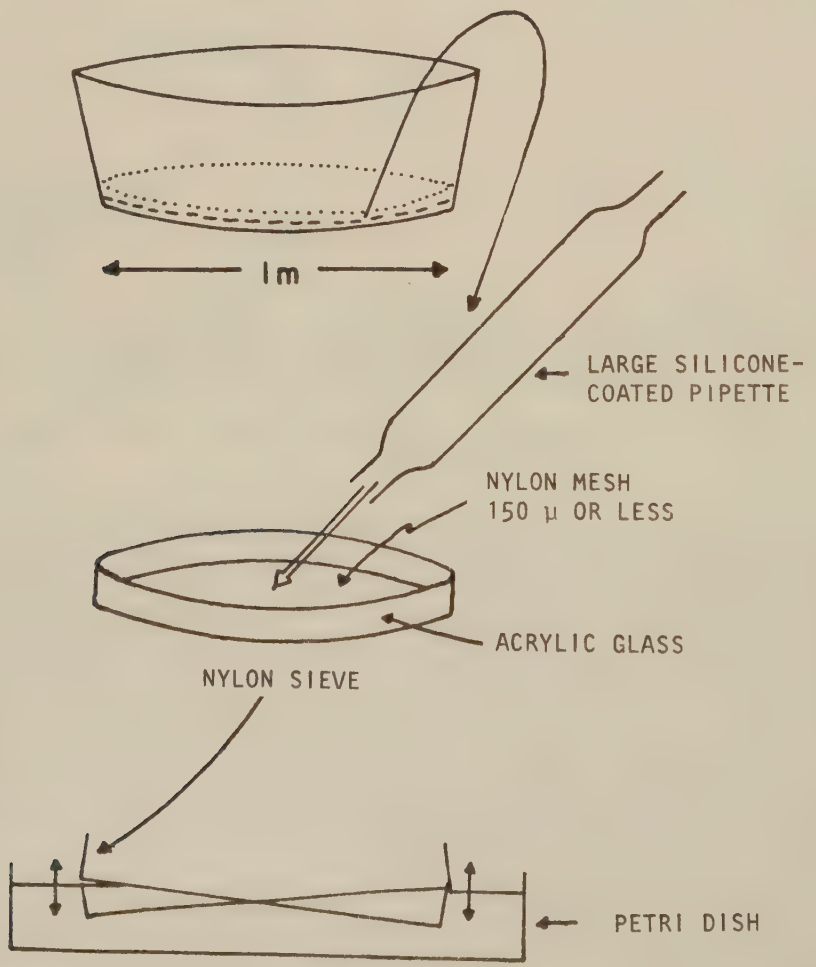


Figure 19. Swedmark method for extraction of meiofauna
(From Hulings and Gray, 1971, with kind permission of Smithsonian Institution Press, Washington. © 1971)

A number of extraction methods are available from which one or a combination of several must be chosen according to the type of samples and their sedimentary structures. For a review of methods see Holme and McIntyre (1971) and Hulings and Gray (1971). As a rule the treatment of meiobenthic samples is very much easier and much more accurate if live rather than preserved samples are used.

The samples taken from substrate of homogeneous fine sand or mud sediments can be treated relatively easily by the Swedmark method, illustrated in Figure 19 and described by Hulings and Gray (1971). The sample, stirred to break up lumps, is placed in a large vessel and covered with 1 to 2 cm of sea water. The mud surface is then pumped into suspension, using a large silicone-coated pipette[1/], and transferred to a nylon sieve or series of sieves (250 µ, 62µ), the largest having a diameter slightly less than the normal size of the petri dish used. Sieving is done by gently rocking the sieve in sea water, either in another vessel or in the original one so that the filtrate is returned to the original sample. When this is complete the sieve is placed in sea water in a petri dish so that the fauna can be examined under a binocular microscope before being transferred from the sieves, when they are likely to be damaged.

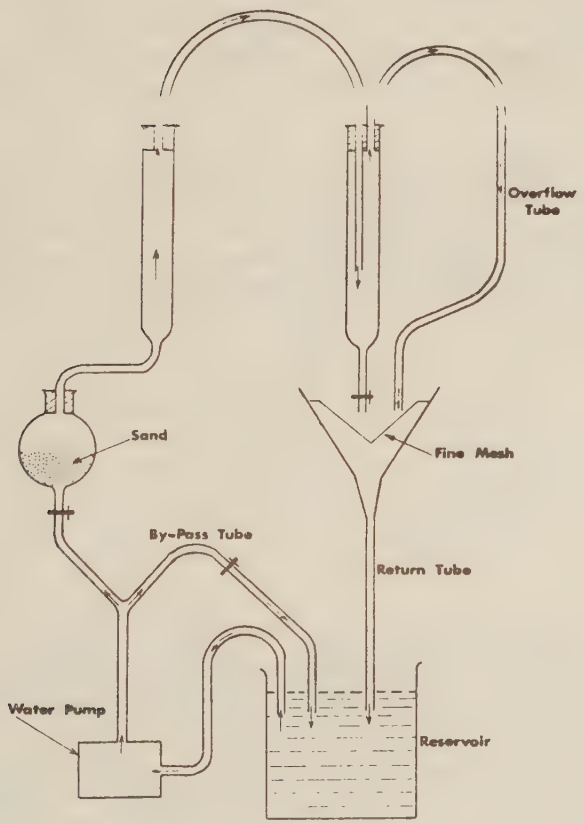


Figure 20. Boisseau type apparatus for elutriation of meiofaunal samples, closed-system arrangement
(From Holme and McIntyre, 1971, with kind permission of the International Biological Programme, London. © 1971)

[1/] The inside of a clean pipette is coated by forcing a 1 : 100 dilution of a water-soluble silicon (e.g. "Siliclod") in distilled water. After complete immersion, the pipette is rinsed thoroughly with water and air dried for one day at room temperature.

For meiobenthic samples of coarser and sorted sand (true interstitial meiofauna) the Boisseau elutriation method in closed-system is recommended (Fig. 20). This method is more sophisticated but not time-consuming. According to Hulings and Gray (1971) the sample is placed in the separation funnel and an equal volume of 6 percent $MgCl_2$ solution is added (for anesthetization of organisms which tend to attach to sand grains). After about 10 minutes, a continuous stream of filtered sea water is introduced through the tap on the separation funnel. After 15 minutes of elutriation, the tap on the tube above the filter is opened and the water allowed to drain through the sieve (50 to 70 µ mesh). The sieve is inverted in a petri dish and the meiofauna washed off with a jet of filtered sea water. A large part of the light fauna will be collected on the sieve, but heavier organisms, such as molluscs, ostracods and foraminifers, might remain in the sediment residue so it has to be examined microscopically. This method can also be satisfactorily used for the elutriation of preserved samples; in this case an open-system of a continuous stream of sea water can be applied, only the incoming sea water must first pass a filter in order not to contaminate the sample.

For the treatment of very heterogeneous samples, such as those obtained on hard or marl-detritic bottoms, a convenient, although quantitatively incorrect, method is the seawater-ice technique described by Uhlig et al., 1973 (see Fig. 21). The sample is placed at the lower end of a large plastic tube tightly covered by 120 to 150-µ mesh nylon gauze, which just dips into filtered sea water in a collecting dish. The sample is covered by a layer of cotton wool, and the tube is filled with the crushed seawater ice. As the ice melts, motile meiofauna move through the gauze into the collecting dish due to salinity/temperature gradients and the streaming action of the water of different densities. If the samples to be treated contain a significant amount of mud, silt or clay, it is advisable to wash them on a 50 to 70 µ screen before this treatment. Obviously, only living samples can be processed by this method.

As mentioned, the final separation and sorting of meiofauna can be done only under stereoscopic microscope. The best type of sorting vessel is a medium-size petri dish, with marked lines on its outer bottom for better orientation while scanning both this and the surface covered by the sample. For separation of organisms, capillary pipettes, fine needles, loops and watchmaker's forceps are needed. In order to make them more clearly visible, and to differentiate biota from detritus and sediment particles, treated samples should be stained with Rose Bengal after being preserved. For this purpose, 10 ml of the stock solution (1 g stain powder/100 ml ethanol) is added to 100 ml of sample plus preservative.

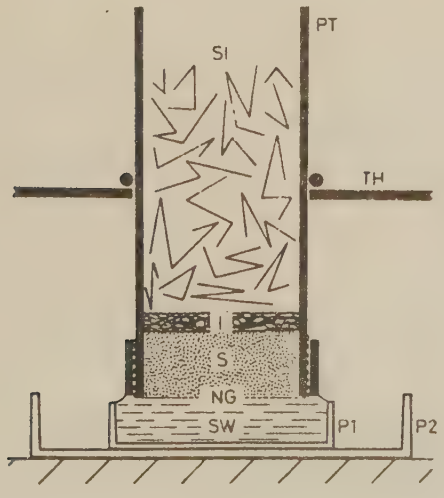


Figure 21. Uhlig's method for the extraction of meiofauna
I - insulation material; **NG** - nylon gauze; **P1**, **P2** - petri- or culture dishes; **PT** - plastic tube; **S** - sediment; **SI** - seawater ice; **SW** - sea water; **TH** - tube holder
(From Uhlig et al., 1973, with kind permission of Biologische Anstalt Helgoland, Hamburg. © 1973)

2.4.5. Preservation, labelling and storage of samples

For practical reasons the most common procedure is the fixation and preservation of benthic organisms in total samples after being sieved or otherwise extracted from the bulk of a substrate. As suggested above (see 2.4.2.2) all material which remains on screens is quantitatively transferred into a tray and then into a suitable jar. Sea water is added almost to the top of the jar and the volume of sample + sea water estimated. The required amount of concentrated formalin (= 38-40% formaldehyde) is calculated so that its final formaldehyde concentration in the jar will be 2 percent (about 50 ml concentrated formalin/litre of water+ sample), and that amount is added to the jar. In order to prevent the formalin solution from becoming acidic, a buffering substance such as hexamine is added, and the whole content gently but thoroughly mixed. About 8 g hexamine/litre of 2% formaldehyde solution is adequate for neutralization.

Large and heavy organisms or bigger mineral particles should be removed from screens or trays and preserved in separate containers, and not with the bulk sample, in order to prevent possible damage of delicate organisms. If time and facilities permit, it is also suggested that some taxonomic groups be processed separately because of specific fixation needed for morphological observations and taxonomic identification. Some organisms (such as actiniarian and other "soft" cnidarians, turbellarians, opistobranchian and other molluscs without exoskeleton, nemertines, echiurids, priapulids, sipunculids and enteropneusta) contract in fixation/preservation liquids and entirely change their natural body form. Therefore, it is better to transfer some specimens alive from the total sample into jars with sea water in which an anaesthetic substance is gradually added, usually $MgCl_2$ up to 4 percent concentration, or some menthol crystals. After a certain period such organisms usually become fully expanded and prepared for transfer into a fixative where they no longer contract due to anaesthetization. The 5 to 8 percent formalin (= 2-3% formaldehyde) in sea water is the most common fixative also for the majority of these groups; however, for those whose identification necessitates histological preparations (for instance, turbellarians, nemertina, etc.) warm Bouin's fixative is more suitable (for preparation see a histological manual). Some macroalgae, sponges, cnidarians and bryozoans have delicate body structures which can be quickly destroyed if preserved within the bulk sample. Although they can be fixated and preserved in the ordinary way, it is better for this reason to preserve them in separate jars.

Most taxonomic groups can be definitely preserved and stored in the same solution as primarily used for fixation, i.e. 5 to 8 percent formalin in sea water, except those with delicate calcareous skeletal structures whose morphology should remain completely intact for the needs of taxonomic identification. These are calcareous algae, foraminifera, sponges, madreporaria, gorgonids, small-size molluscs, serpulids, bryozoans, etc. Therefore, it is recommended that at least these groups are sorted out from bulk samples as soon as possible, i.e. within one month after sampling, and preserved in 70 percent ethanol, because even carefully neutralized formalin solutions tend to become slightly acidic, dissolving fine calcareous structures.

There is usually a considerable lapse of time between the sampling, sorting and taxonomic identification, and consequently samples must be held in bulk storage. It is therefore imperative that samples be stored in suitable containers which prevent evaporation of preserving liquids, caps of jars and vials must be airtight; in addition, it is advisable to line the rim of a container with an adhesive plastic tape. Samples must be kept in a dark place at room temperature and they should be periodically checked for evaporation and pH in preserving liquids.

It is imperative that samples for any purpose and any specimens removed or sorted out from them have adequate labels bearing all necessary data. Although it is practical to write basic information (station number, sampling date, etc.) on tops or sides of the jars with a waterproof marker, a label must be placed inside the jar immediately after the sample is collected or sorted. The labels for bulk samples must be strong enough to withstand both preserving liquids and abrasions made by sample particles during transport. Sheet plastic labels with a matt surface for writing with a hard pencil are the most suitable for this purpose. For sorted and deposited samples, any strong waterproof paper can be used, ideally a goatskin parchment paper, on which information is written in hard pencil, china ink or typed with a water- and ethanol-proof ribbon. The minimum information required on the label, at least for the primary sample, is the following:

- sampling institution
- sampling programme
- research vessel
- family name of collector
- sampling station: number, geographical coordinates, depth
- date and hour of sampling
- sea conditions during the sampling
- sampling gear used and sampled surface
- estimated volume of the sample
- description of the treatment: method, screen mesh, preservation
- description of the substrate
- description of the sample and specific notes (reverse side of label).

In addition to the label information, records with detailed notes and available environmental and ecological data should be made and retained in permanent files which are subsequently completed with the data on sorting, taxonomic identification, determination of abundance, density of species population and biomass.

2.4.6 Description of biomass

Biomass is considered here as the weight of a partial population of a species or a composite group of species which, at a given moment, inhabited a sampled surface or volume. For convenience and comparability, the biomass may be reported per larger units, e.g. per 1 m^2, as calculated from the mean of replicate samples within the subarea of an investigated station or even for multi-station subareas.

Biomass can be expressed in units of:

- wet weight;
- whole or decalcified dry weight;
- ash-free dry weight, i.e. approximate weight of organic matter;
- carbon and/or nitrogen content;
- caloric value.

Although from the standpoint of functional bioproductivity and matter and energy flows in ecosystems the last two measurements are the most appropriate ones, for practical reasons, and considering immediate needs of investigations focused on community structure, only the first three, rather crude, measurements are discussed in detail and recommended in the manual.

2.4.6.1 Wet weight

This is the most crude measurement of the biomass and can be done even on small research vessels or elsewhere in the field, preferably before specimens are preserved. The most suitable instruments for this purpose are topload or spring balances unless samples are so minute that an analytic balance, which cannot be used at sea, is required. Specimens to be weighed must be free of attached sediments and detritus particles, water should be mopped up from body surfaces with blotting paper and the water from external cavities (e.g. mantle cavity of molluscs and ascidians, oscula of sponges) emptied. For larger species (>1 cm) with heavy exoskeletons, the biomass is often determined only for soft parts, while the mineral part is removed (e.g. shells of molluscs and hermit crabs, tubes of polychaetes). This approach is not recommended, since it is non-systematic because smaller species of

the same taxonomic groups cannot be treated in this way, and for some large organisms it is just not feasible (e.g. calcareous algae, madreporaria and bryozoans). However, if it is applied, then this should be clearly reported in the records.

2.4.6.2 Dry weight

This is the weight of totally dehydrated organisms at the moment when it becomes constant. There are several methods of drying, such as by low-temperature desiccators or lyophilization and by the heat of infrared rays or ovens. The latter still seems the most practical and is widely used. The drying temperature in the oven is ideally set at 70°C; higher temperatures up to 100°C may be selected too, especially for bulky material, but it should not exceed 105°C in any circumstances. The duration of drying up to the stage of constant weight is very variable, depending on the type and amount of biological materials. Standard macrobenthic samples usually require 24 hours. Some maceration of megabenthic organisms and gentle ventilation in the oven can speed up drying operations if necessary. As samples are dried in the vessels in which they will also be weighed, these vessels should be carefully cleaned, dried and tared at the same balance as that used for biomass determination. In order to avoid time-consuming transfer of samples into other types of vessels for the ashing procedure after their dry weight is determined, the same vessels should be used for both operations, i.e. silicon or heat-resistant ceramic crucibles. Precision top-load balances are used for large samples and analytic ones for small samples with $\pm$ 1 percent reproducibility. For meiobenthic samples a microbalance is required.

Specimens to be dried and weighed are prepared as described for wet weight above. In addition, they must be completely free of sea water and therefore should be washed in fresh water, ideally distilled water, prior to drying. Calcareous skeletons may be elimited, either mechanically or by chemical decalcification. These procedures are not recommended for reasons already discussed, and because more accurate results are obtained by ashing as described below.

2.4.6.3 Ash-free dry weight

Samples whose dry weight has been determined are transferred into a muffle furnace (with a stable thermoregulation and equipped with a thermorecorder, if possible), where all organic matter is burnt off at 500°C within 6 to 12 hours, depending on the amount and consistence of samples. When incineration is completed, the samples are cooled down to about 80°C in an open place first and then to room temperature in a desiccator, and weighed. Analytical and microbalances must be used. Ash-free dry weights are obtained by subtracting ash weights from dry weights. For samples composed mainly of soft-body organisms, the ash-free weight presents a reliable measurement of total organic matter. Unfortunately, this is not true for samples with a high percentage of calcareous skeletons because during the incineration considerable volatilization of inorganic carbon dioxide from carbonates occurs. Consequently, the ash content is underestimated and the obtained values for organic matter are too high. In such a case some calibration procedures are recommended (see Holme and McIntyre, 1971).

Biomass measurements are best made with fresh or deep-frozen samples. If this is not possible, formalin-preserved samples can be used, though with care, and not before they have been preserved for three months, because of unpredictable changes in relative weight of preserved organisms before the final stabilization. Ethanol-preserved samples should not be used for biomass estimation because a considerable amount of organic matter is lost due to dissolution in extraction solution.

SUMMARY

The following processing of benthic samples is recommended:

(a) As soon as possible after samples are obtained, i.e. when the majority of biota is still alive, they are carefully sieved, optimally by Sanders' technique, over a screen of 1.0- or 0.5-mm mesh with the help of seawater flushing.

(b) The extraction of meiofauna requires special procedures such as flotation, centrifugation and elutriation (see 2.4.4.3). The remainder is quantitatively transferred into jars, fixated and preserved in 5 percent neutralized formalin solution in sea water and carefully labelled. The sorting and taxonomic analyses are performed later on in the laboratory. Where the analysis of samples must be postponed for longer periods, it is recommended that a 70% ethanol solution be used instead of the formalin solution.

(c) In the laboratory all biota is manually sorted from mineral and/or detritic residues into taxonomic groups to facilitate identification at species level (see 2.2). For macrobenthos at least, the sorting must be done under a dissecting stereomicroscope.

(d) The biomass can be determined at species and/or group level using the same material as for species identification and abundance enumeration; however, it is best to have parallel samples, biota extracted alive and preserved deep-frozen. The procedure recommended is to dry samples until constant weight at 70°C, then the dry weight is recorded, the samples incinerated at 500°C, the ash weight recorded, and by calculating the difference the biomass is obtained.

2.4.7 Taxonomic identification and enumeration of species abundance

As already suggested (see 2.2), an operating research team should include specialists for the identification of those crucially important taxonomic groups which can be realistically covered by an average marine laboratory: macrophytes, molluscs, echinoderms, decapods and, tentatively amphipods, as well as foraminifera and harpacticoids, if dealing intensively with meiofauna. Some groups, which are usually represented by a smaller number of easily recognizable species, can be identified by non-specialist members of a research team; but there are a number of taxa for which specialists have to be found elsewhere. For such groups the total composite abundance can be determined and wet-weight biomass estimated after all specimens have been preserved, labelled and shipped to specialists or deposited.

The above-mentioned major groups are identified at species level, or just temporarily coded where a species is clearly recognizable, but the definite nomenclature requires further research (for example Capitella sp. No. 1, Capitellidae gen. No. 1, sp. No. 1). During the identification procedure or afterwards, depending on individual practice, the abundance of all species found in the sample is enumerated and recorded. Obviously only the live specimens in the sample are considered, not the skeletal or other remnants. In the case of damaged specimens (usually polychaetes and decapods) only the heads are counted as specimens, not the abdominal parts or appendages.

There are not many problems with the determination of species abundance for soft bottom and for the vagile and solitary sedentary organisms of hard bottom samples. However, for densely clustered smaller species of algae, hydroids, anthozoans and encrusting calcareous algae, sponges and bryozoans, the estimation of abundance is impossible; only the relative surface cover can be estimated as a measurement of their quantitative contribution in the community, and of course of the biomass. In order to get reliable information, the relative cover should be determined as much as possible in the field while collecting samples. Also the samples should be taken and preserved in such a way as to produce least modified distribution of sedentary biota on a hard substrate, and to facilitate the laboratory determination of the relative cover made by smaller organisms. All data on species composition, abundance and relative cover must be recorded separately for each analysed sample.

2.4.8 Compilation of results and identification of distinct communities

With all data recorded, relative abundance and relative biomass for individual samples are computed and then the mean absolute, as well as relative abundance, cover and biomass with relevant standard deviations are calculated for the total samples taken at individual stations. Individual or composite sample diversity indices can be computed and recorded as suggested below.

In order to obtain summarized information on the composition of those benthic assemblages which might present distinct communities, the recorded are divided into groups of presumably similar stations, selected on the basis of similar environmental conditions and on first impression of the biocoenotic composition recorded during sampling. Then the real biocoenotic compositions of all pairs of grouped stations are compared by a similarity analysis or other method, showing which stations are apparently taken from a homogeneous patch of distinct community. A further compilation can be made of all samples taken at such highly similar stations to obtain to most reliable basis for any interpretation of the composition of benthic communities, and particularly for the measurements of diversity as a significant parameter of pollution-induced biocoenotic modifications.

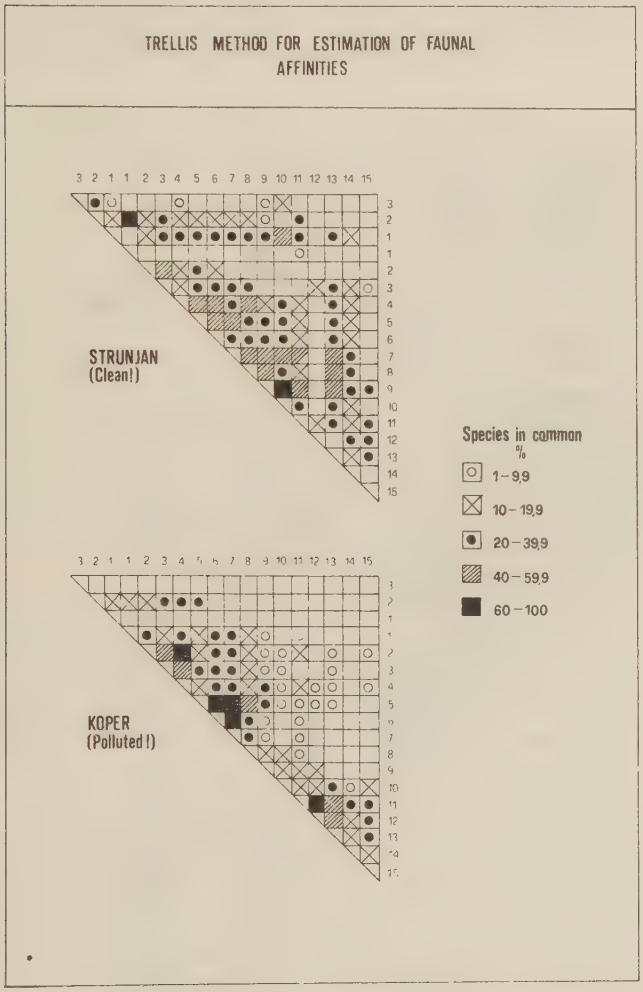


Figure 22. Trellis interpretation of species composition affinities of samples (From Stirn et al., 1975, with kind permission of Pergamon Press, Ltd., Oxford. © 1975).

There are a number of methods of analysis of similarity, i.e. affinity between biotic composition of samples. For quick routine procedures a convenient method is the calculation and graphic presentation of affinities between compared samples within a trellis diagram (see Figure 22) as given by the coefficient of community (CC) in Jaccard (1928):

$$CC = \frac{Cij}{Ai + Aj - Cij}$$

where Ai(j) = number of species in sample i(j); Ci(j) = number of species common to both samples i(j). Thus CC x 100 is the measurement of the percentage of species shared by two samples. For the majority of benthic assemblages Sanders' index of affinity is even more reliable as it is sensitive also to differences in the relative abundance of species in the two compared samples. According to Sanders (1960) its value is obtained by a matrix method, the trellis diagram (see Figure 23) from percentage composition of the various species in each sample. The samples are arranged at right angles along the ordinate and abcissa and all possible pairs of samples are compared for their faunal content. The resultant value, the index of affinity, is a measurement of the percentage of the fauna common to a pair of samples and is obtained by totalling the smaller percentage of those species present in both samples. For example, if species X represents 10 percent of sample A and 20 percent of sample B, 10 percent of the total affinity between the samples would be attributed to species X. The table is then rearranged so that the samples with the highest values are brought into close proximity. In this way the samples that are most ecologically alike are grouped together. Sanders's index could be based on either relative abundance or biomass.

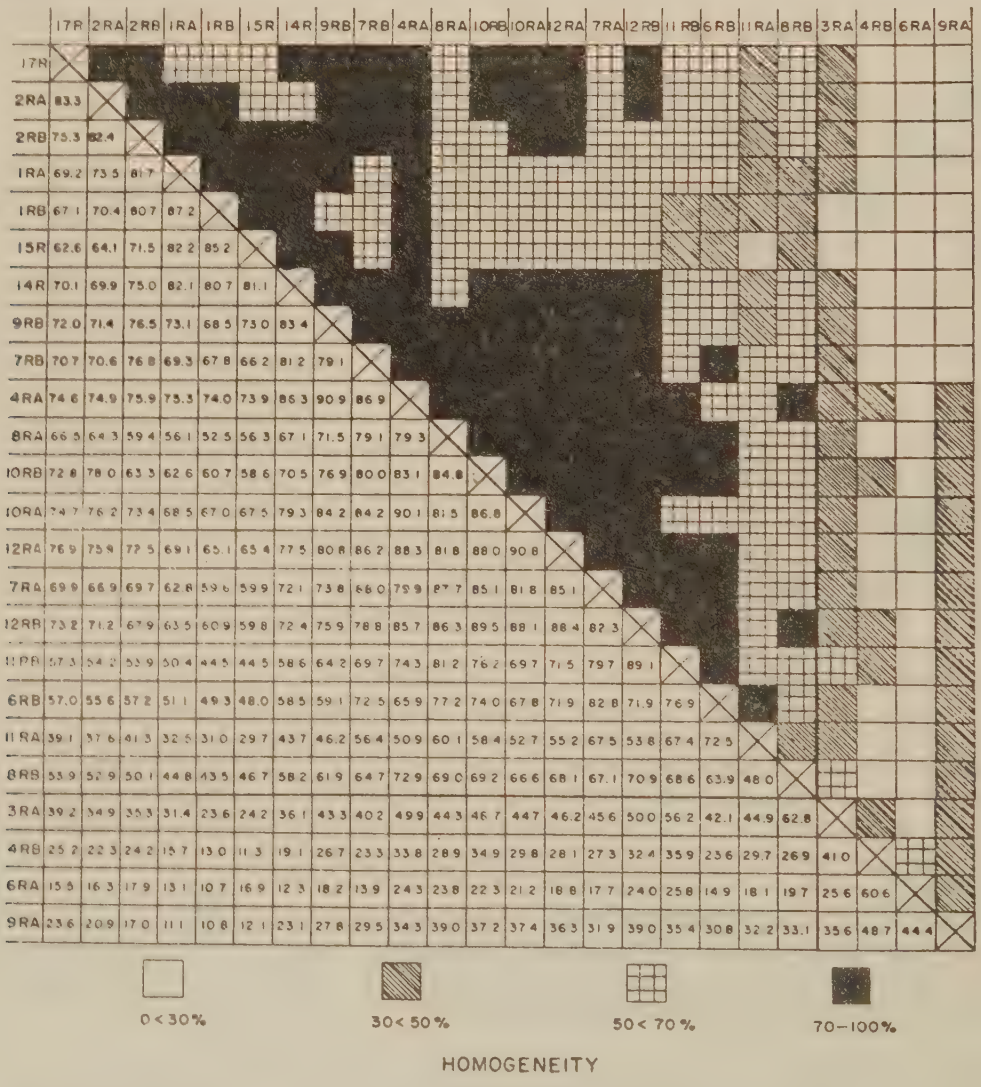


Figure 23. Trellis interpretations of species composition affinities of samples (From Sanders, 1960, with kind permission of the American Society of Limnology and Oceanography. © 1960)

The above measurements of similarity and homogeneity, respectively, present not only a way to find out which stations were taken from the patch of a distinct community but they are also an indication on whether the replicate random samples were taken properly at an individual station.

Low similarity between replicate samples mostly indicates an inadequate sampling or, exceptionally, a very heterogeneous benthic assemblage within the subarea of an individual station. Therefore, the calculation and recording of CC or Sanders' index for all pairs of collected samples is strongly recommended.

2.4.9 Interpretation of structural features of benthic communities as a measurement of pollution-induced modification

On the basis of suitably analysed and compiled data, it is fairly easy to make descriptive and graphic interpretations of some apparent characteristics of communities identified by the methods suggested above. Within the investigated area at least some patches of the same distinct communities are certainly distributed in both polluted or suspected as well as in "clean" subareas. Such patches can be compared from various aspects, such as:

- species composition and their abundance;
- quantitative composition by species abundance, cover or biomass;
- dominant species and identification of the types of communities characterized by the taxa of the first rank dominant organisms;
- rough estimation of semi-quantitative relationships among the major trophic types of organisms, such as total primary producers, ratios between green, red and brown algae, deposit feeders and suspension feeders, and ratios between omnivorous and specialist types, and of the relative importance of specific and omnivorous predators;
- distinct disappearance of some species normally present in non-polluted subareas of certain communities, and relative increase in the size and biomass of some tolerant autochthonous or recently immigrated species;
- other indications of significant changes in the above aspects in the polluted or suspected subareas of distinct communities as compared with the conditions in the "clean" subareas and/or in similar pollution-free areas.

Although the structural features of communities and their pollution-induced modifications are usually quite apparent, their statistically correct quantification is rather difficult and complex. Also the theories of relevant aspects of mathematical ecology are not yet developed to the stage where they can offer fully reliable methods for routine analysis of communities (for more details on the subject see Boudouresque (1971) and Pielou (1969)).

For the time being, therefore, of the possible quantitative measurements of bicoenotic structural features only the diversity approach is suggested for pollution-oriented studies of benthic communities. The justification of this diversity approach is based on the working hypothesis that the community structure provides the most significant reflection of pollution-induced modifications of ecosystems. A community is defined in this context as an association of interacting populations of species inhabiting a given environment at a given time; it is characterized by a level of adaptation and homeostasis which is determined in part by the duration of the evolutionary history of the ecosystem. According to this definition, the most stable and usually the most diverse communities are derived after long periods of evolution in an ecosystem whose environment is characterized by a low level of fluctuation in its physico-chemical parameters in space and time, and by generally favourable conditions of life for the largest number of species. Considering communities in polluted ecosystems from this standpoint, their stability and diversity must obviously be relatively low for several reasons. Time, as a basic condition for the development of stability and homeostasis, has been relatively non-existant for polluted ecosystems, which are features of the modern world. Therefore, communities in polluted environments may be considered as the most characteristic examples of the "immature communities" described by Margalef (1968). Even if we could somehow ignore the speculative consequences of the time factor, conditions in polluted ecosystems are obviously far from favourable to the development of stability, even in the case of the least directly destructive pollution influence, that of sewage discharge or other wastes consisting of biodegradable organic matter.

Organic effluents, as still the most common polluting factor, introduce into marine ecosystems a complex of primary effects which can be divided into two basic categories, requiring almost opposite ecological considerations:

(i) Nutrient supply, with a more or less continuous high rate of input both for primary producers and for elements of higher trophic levels. Initially this might be considered a beneficial influence, particularly in oligotrophic systems. However, in many circumstances secondary consequences may arise from eutrophication to the development of anoxic conditions, whose intermediate and final consequences are at least as destructive to ecosystems as those from the second category (Štirn, 1971).

(ii) Introduction of toxic and inhibitory factors from organic effluents, leading to the extermination of intolerant species. This involves a complex spectrum of influences. However, a number of the most negative effects can easily be recognized, such as decreased salinity, pH, oxygen level and transparency; increased turbidity and sedimentation rates, carbonic acid level and BOD; plus the presence of directly harmful materials such as various intermediate products of protein decomposition (particularly amines, mercaptanes, H_2S and NH_3) and residual components of organic effluents such as pesticides, heavy metals, phenols, bacteriostatics and antibiotics. Finally, it should be stressed that all the above-mentioned factors are subject to extremely frequent and violent oscillations in their composition and rates of input, presenting a decisive stress in an ecosystem. This is true even in the case of oscillations of relatively harmless factors such as temperature and salinity.

Under these conditions, the most drastic initial modification of a polluted ecosystem is the inevitable extermination of non-tolerant species or even entire taxonomic groups such as sponges, most of the cnidarians, the gastrotrichs, kinorhynchs, echiurids, sipunculids, stomatopods, cumaceans, scaphopods, most of the echinoderms and the ascidians. Considering the dynamic equilibrium of ecosystems on the one hand, and the above-mentioned nutrient supply on the other, the loss of species would necessarily be followed by a vast and complex process of dislocation in the total food web of the polluted ecosystem, reaching its peak in highly eutrophic and anoxic conditions. This process is characterized basically by hyperproductivity of certain tolerant primary producers, the dominance of deposit feeders and certain tolerant suspension feeders, and the suppression of most carnivore and herbivore macrofeeders. The end result is thus most significantly expressed in extraordinarily high amounts of unutilized organic matter, and their subsequent deposit in the ecosystem, plus the structural simplification of the polluted community (see Štirn, 1971).

The above simplification of community structure begins, as a primary effect of the introduction of effluents into the community environment, with species extermination, the rate of extermination being proportional to the rate of pollution and reaching a maximum (i.e. minimum number of species) in extremely eutrophic conditions. On the other hand, due to increased nutrient supply and dislocations within the food web, the abundance and biomass of the remaining super-tolerant species tends to increase enormously in the system.

Thus, the most logical index of the rate and degree of such pollution-induced modifications would seem to be community structure, expressed as the relationship of numbers of species to abundance or biomass - that is, biotic diversity. The value of diversity as an index of the influence of organic pollution was provided by testing various methods, as reported by Štirn et al. (1975). However, since other, directly toxic, forms of pollution also seem to result in community simplification, the application of diversity indices can probably be justified in the general detection and assessment of marine as well as freshwater pollution. Furthermore, in the author's opinion, mankind will soon be forced, by further threats to his continued existence on earth, to change his concept of "pollution" from the anthropocentric "beneficial uses" definition to the ecological justified definition of pollution by Patrick (1950) as "anything which brings about a reduction in the diversity of aquatic life and eventually destroys its balance". Therefore, the diversity index should be considered as one of the decisive parameters not only in scientific but also in legal studies and environmental impact statements, regardless of the methodological problems which, in any case, are not more difficult than those facing chemists, bacteriologists and toxicologists.

Although the available methods of measuring biotic diversity are still far from perfect, and a source of much disagreement among theoreticians concerning the relative adequacy of various mathematical expressions as well as their true theoretical and practical significance, for the purpose

of pollution studies some of these methods are certainly of great immediate value. The most common are reviewed below and technical details given on those recommended.

2.4.9.1 Interpretation by species-area curves

This method is probably the oldest graphic expression of biotic diversity; it was widely used by the classic phytocoenologists (Arrhenius, 1918; Gleason, 1922, and others) and introduced for marine pollution studies by Štirn (1965, 1970). It is based on the fact that generally the larger the area of samples the larger the number of species found. This method is carried out by collecting a progressively larger number of samples within a given area, and thus obtaining more comprehensive examinations of a greater number of microhabitats in order to yield additional species other than the dominant and perhaps accidental ones found in the initial samples. Regardless of the number of individuals, only the number of species found in individual samples are plotted against the progressively larger number of samples, as shown in Figure 24. The curves C_1 -C_3, K_1, K_2, represent the typical pattern for uniform communities in heavily polluted ecosystems in the Gulf of Trieste and the Lake of Tunis, where enlargement of the sample does not include additional species. This situation is compared to more typical Arrhenius' curves, C_4, C_5, S_1, S_2, for communities in clean or only slightly polluted environments where samples include progressively rarer species. Although this method is a rather simple one, and is strongly dependent on the particular habitat and selection of sampling gear, it can be recommended for orientative studies (for technical details, see Štirn, 1970).

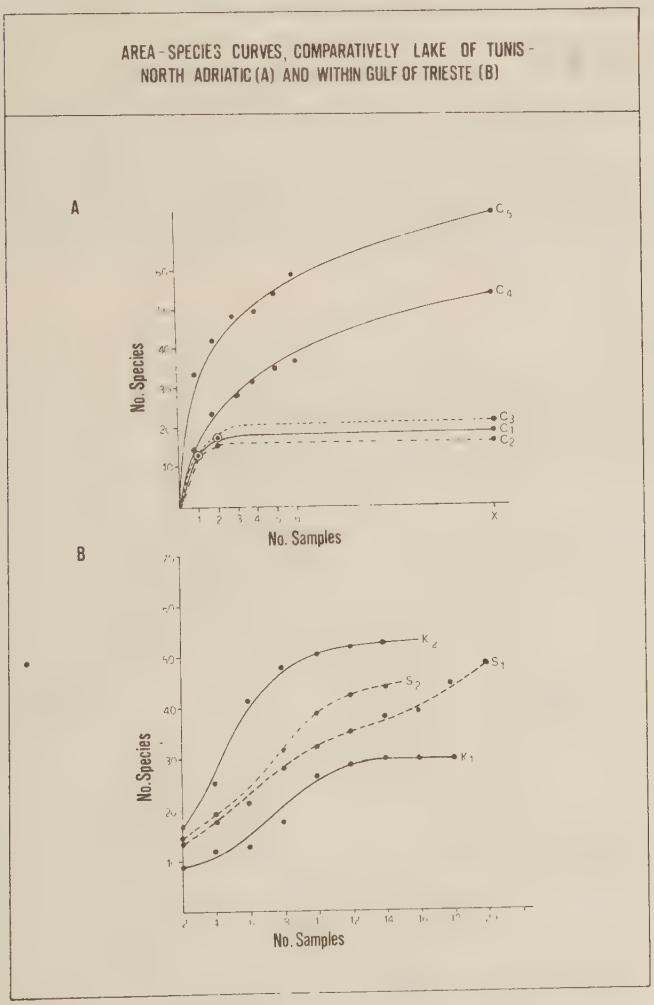


Figure 24. Various types of area/species curves from "normal" (curves C_4, C_5, S_1) and pollution or estuarine "stress communities" (curves C_1-C_3, K_1, K_2 and S_2)
(From Štirn et al., 1975, with kind permission of Pergamon Press Ltd., Oxford. © 1975)

2.4.9.2 Interpretation by rarefaction diversity curves

This method was developed by Sanders (1968) and widely adopted by ecologists for the assessment of biotic diversity of normal ecosystems. According to results (Štirn et al., 1975), of all available expressions of diversity, this seems to be the most acceptable one for use in pollution studies, due to the fact that it is relatively independent of the sample size, particularly for communities in which organisms are rather randomly or evenly distributed. Sanders' rarefaction curve is a graphic representation of a continuum of relationships between number of individuals and number of species, starting from a large actual sample for which the data on abundance and species composition has been determined. These data are deductively rarefied, giving the reduced number of species to be expected in progressively smaller samples (an opposite process to that used in species/area curves) under the assumption that the ratio between numbers of individuals and species remains the same. The resulting curve is thus a curvilinear interpolation of the species abundance found in the total sample and can be considered as a constant property of the community from which the sample was taken.

The necessary computations for obtaining data for the construction of a rarefaction curve are made from actual data on species and individuals from two sample sites (Avčin et al., 1973), demonstrated in Table I and II. Species are first ranked by their abundance and percentage composition of each species, and cumulative percentage is given. The following example is based on Sanders' text, but uses data from Table I and II. The percentage composition in the calculations is the same as in the actual sample, but the number of individuals is reduced to 100. Since 100 specimens in this reduced sample represent 100 percent of the individuals present, then each individual specimen represents 1 percent of the sample. In the actual sample, five species each comprise 1 percent or more, and in total they comprise 97.5 percent of the sample by number. Therefore, each of these five species will be present in the reduced sample. This leaves a residue of 2.5 percent of the actual sample comprising the remaining 9 species. Because none of these species forms more than 1 percent of the actual sample, those species of this group that will appear in the reduced sample cannot be represented by more than one individual since one specimen comprises 1 percent of the reduced sample, then 2.5%/1% = 2.5 species; 3 + 2.5 = 7.5 species present per 100 individuals. In order to obtain enough data for the constructions of a rarefaction curve, numbers of species are computed for large numbers of individuals (25, 50, 100, 150, 200, 300 ...) in the same manner. The resulting number of species, plotted against the above numbers gives the smooth curve shown in Figure 25.

Table I

Example for preparation of data in the construction of species diversity curves by Sanders' rarefaction method: Assemblage of heavily polluted community in the Bay of Koper, Stations KA 1-4 (8 grab samples grouped as 1 large sample)
(From Štirn et al., 1975, with kind permission of Pergamon Press Ltd., Oxford. © 1975)

Rank of species by abundance	Number of individuals	% of sample	Cumulative sample %
1	848	57.4	57.4
2	357	24.2	81.6
3	194	13.1	94.7
4	24	1.6	96.3
5	17	1.2	97.5
6 - 8	8 each	0.5 each	99.1
9	3	0.2	99.3
10 - 14	2 each	0.1 each	99.99
Total number	1 477		

Table II

Example for preparation of data in the construction of species diversity curves by Sanders' rarefaction method: Assemblage in clean environment in the Bay of Strunjan, Stations SA 1-4 (8 grab samples grouped as 1 large sample) (From Štirn et al., 1975, with kind permission of Pergamon Press Ltd., Oxford. © 1975)

Rank of species by abundance	Number of individuals	% of sample	Cumulative sample %
1	76	38.9	38.9
2	22	11.3	50.2
3	14	7.2	57.5
4	12	6.2	63.7
5	11	5.6	69.3
6 - 7	8 each	4.1 each	77.5
8 - 10	6 each	3.1 each	86.8
11 - 12	4 each	2.1 each	91.0
13 - 21	2 each	1.0 each	100.0
Total number	195		

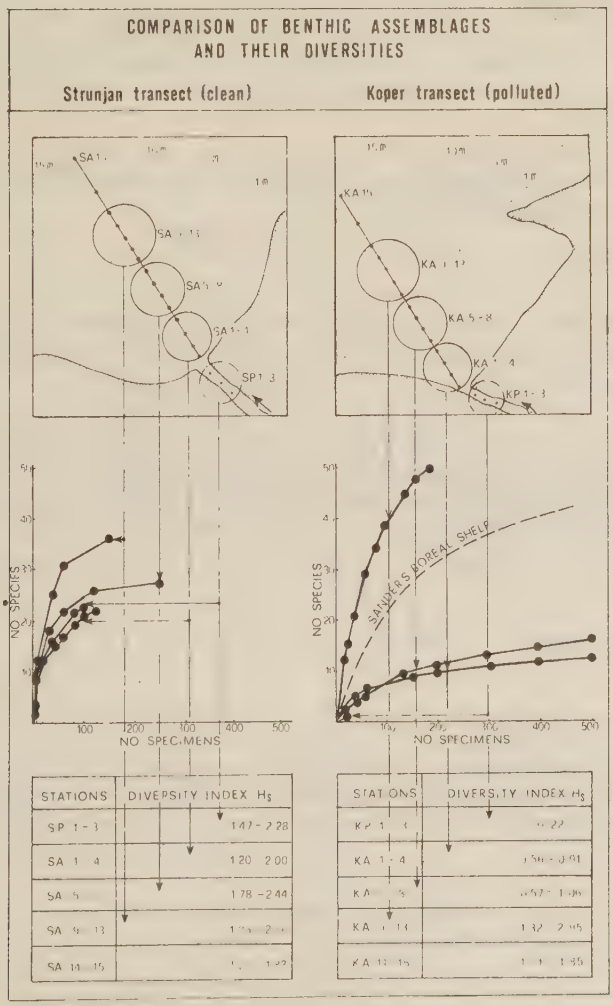


Figure 25. Rarefaction curves and Shannon-Weaver diversity indices obtained for the physically comparable bays of Strunjan and Koper, the latter being heavily polluted (From Štirn et al., 1975, with kind permission of Pergamon Press Ltd., Oxford. © 1975)

Sanders' method was tested quite successfully, and polluted communities showed very characteristic curves. Although this method requires much care in the preparation of the sampling programme and much sorting, identification and data manipulation, it is strongly recommended for pollution studies.

2.4.9.3 Interpretation by diversity indices

Besides the graphic expressions of biotic diversity, there are in mathematical ecology several numerical measuments, called diversity indices, proposed by various authors (for a review, see Pielou, 1969). Considering the theoretical approaches which lead to corresponding mathematical expressions, diversity indices may be classified as follows:

(i) Richness indices, based on the number of species present in a given sample or community. Having (s) for number of species found in progressively larger number of samples and (S) for the total number of species found, the species richness index can be expressed by the application of information analysis as:

$$H_S = -\sum \frac{1}{S} \ln \frac{1}{S}$$

Presuming that all existing species in a given community have been found, it follows that maximum information is obtained, thus:

$$H_S = H_{max} = \ln S$$

Therefore, when there is comprehensive knowledge of all species in a system (which, in fact, occurs very rarely), the biotic diversity may be expressed simply from the total number of species.

(ii) Evenness indices, depending primarily upon the patterns of quantitative distribution of individuals among species. Simpson's index is an example:

$$SI = \sum \frac{n_1 (n_1 - 1)}{N (N-1)}$$

where (n_1) = number of individuals of species (i) and (N) = total individuals. Because of the obvious disadvantages of the evenness approach in biocoenological studies of more complex communities, such indices are seldom applied.

(iii) Composite indices, reflecting with more or less equal power of interpretation both aspects of community structure: richness of species and evenness or equitability of individual distribution among species. This relationships may be expressed as the equation for number of cumulative species vs. the logarithm of their abundance, producing diversity indices such as Margalef-Gleason's index (d):

$$d = \frac{S - 1}{\ln N}$$

or Fisher's index (α):

$$S = \alpha \ln (1 + \frac{N}{\alpha})$$

where (S) = the total number of species and (N) = their total abundance in a given sample or community.

It has often been found in practice that diversity indices which are derived from such purely logarithmic functions are in one way or another strongly influenced by the size of a given sample. Therefore, the application of information analysis in the designing of more realistic indices, such as those described below, was welcomed by ecologists. The most commonly applied are Brillouin's index (H):

$$H = \frac{1}{N} \log \frac{N!}{n_1 ! \, n_2 ! \, \ldots \, n_s}$$

and Shannon and Weaver's index (H'):

$$H' = - \sum_{i-1}^{s} P_i \log P_i$$

where (N) = total number of individuals in the sample or community; (s) = total number of species in the sample or community; ($n_1 \ldots n_s$) and (n_i) = number of individuals of (1-s) species; (p_i) = relative abundance of the (i)th species in the range 0.0 - 1.0 = (n_i/n). (log) can be to the base 2, e or 10; most commonly used are natural logarithms, (ln).

Shannon and Weaver's formula seems to be the most consistently useful way of obtaining significant diversity indices which are relatively independent of sample size. As shown in Figure 25 Stirn et al. (1975) obtained about the same results in favour of this index during the testing of the seven most common diversity indices as applied to comparative studies of community structure in clean and polluted habitats. Therefore, the Shannon and Weaver information equation can be recommended as the most suitable mathematical expression of biotic diversity. Practical computations involved in applying this index to data can be seen in Table III.

Table III

Calculation of diversity index
(From Stirn et al., 1975, with kind permission of
Pergamon Press Ltd., Oxford. © 1975)

Species	Abundance	p_i	$(\ln)p_i$	$p_i \, (\ln)p_i$
Capitella capitata	50	0.5000	-0.6932	-0.346600
Podarke pallida	25	0.2500	-1.3863	-0.346575
Corophium volutator	20	0.2000	-1.6094	-0.321880
Cardium edulis	5	0.0500	-2.9957	-0.149785
	100			

Diversity index $H' = - \sum = 1.1648$

A sample of soft bottom was taken, all live macrobenthic organisms sorted out, identified, separated by species and counted. Four species were found; their abundance is shown in column 1 of Table III. Relative abundance, (p_i) = (n_i/N) = species abundance/total abundance, is tabulated in column 2; and its log (ln) in column 3. Column 4 gives the sum $p_i(\ln)p_i$, and the negative sum of these values yields the diversity index (H').

As we have seen, there are certain weak points in the diversity theory. Specifically, it should be made clear that nearly all measurements of biotic diversity are based on the assumption that

the elements of the community are randomly or evenly distributed, which, in nature, is a rare occurrence. So it is important that principles of sampling design and procedures are strictly adhered to.

SUMMARY

After all biota (not skeletal or other residues) is sorted from samples, it must, in principle, be identified and counted at the species level, at least the taxonomic groups (see 2.2). Data on species composition, absolute and relative abundance (or surface cover for encrusting forms), dominance ranks, etc., must be recorded.

For purposes of interpretation of structural features of benthic communities, at least the following analyses should be made:

(i) Similarity measurements for all pairs of sampled stations (considering as stations the integral of all samples taken at a given station) using Jaccard's or, better, Sanders' index of affinity and trellis determination of stations belonging to distinct communities;

(ii) Diversity index for individual samples, combined samples belonging to one station and, for integral of samples/stations, belonging to distinct communities. The use of Sanders' rarefaction method and of Shannon and Weaver's diversity index is recommended

2.4.10 Sampling and analyses of sediments

As mentioned previously, information on environmental conditions for soft-bottom communities must include adequate data on some physico-chemical properties of sediments. It was also suggested that, ideally, sedimentological maps should be prepared by marine sedimentologists before the final set-up of benthic sampling programmes. However, in practice this is rarely done and ecologists have to perform sedimentological sampling and analysis themselves.

For orientation purposes sediment samples can be taken from grab samples immediately after being hauled and from that part where sediments were least disturbed; they can be taken conveniently by manual coring into grab material, using short sections (10 to 15 cm) of tubes as described below. Less valid subsamples are obtained by taking an amount (e.g. 150 ml) of homogeneous benthic material from a grab with a spoon.

Undisturbed sediment samples can be obtained only by coring - by a diver in shallow water, and by a mechanical corer elsewhere. Samples are collected into 50 to 60-cm long tubes (outside diameter 4 cm and inside 3.6 cm, i.e. 10 cm^2 surface of sample) made of transparent acrylic glass, with the tube edge conically sharpened to penetrate sediments.

For mechanical coring operations the Meischner and Rumohr (1974) gravity corer can be recommended. The design and operation are shown in Figures 26 and 27. As can be seen, the tube of the corer is pushed into the sediment like a pipette due to kinetic energy of the free-falling instrument. A sufficient length of free rope has to be paid out on deck before release for free fall, 1 to 5 m above the bottom.

Immediately after the core sample is obtained, the lower opening of the tube is tightly stoppered. The upper end of the tube (interface water-sediment) is cut off 3 cm above the sediment surface and also stoppered. The upper part of the core is marked, the sample labelled and stored in a vertical position. The samples are dried at a maximum temperature of 80°C for standard analysis; for determination of organic substances, they should be preserved deep-frozen.

Some parameters such as pH and Eh as well as description of appearance have to be obtained from freshly obtained samples.

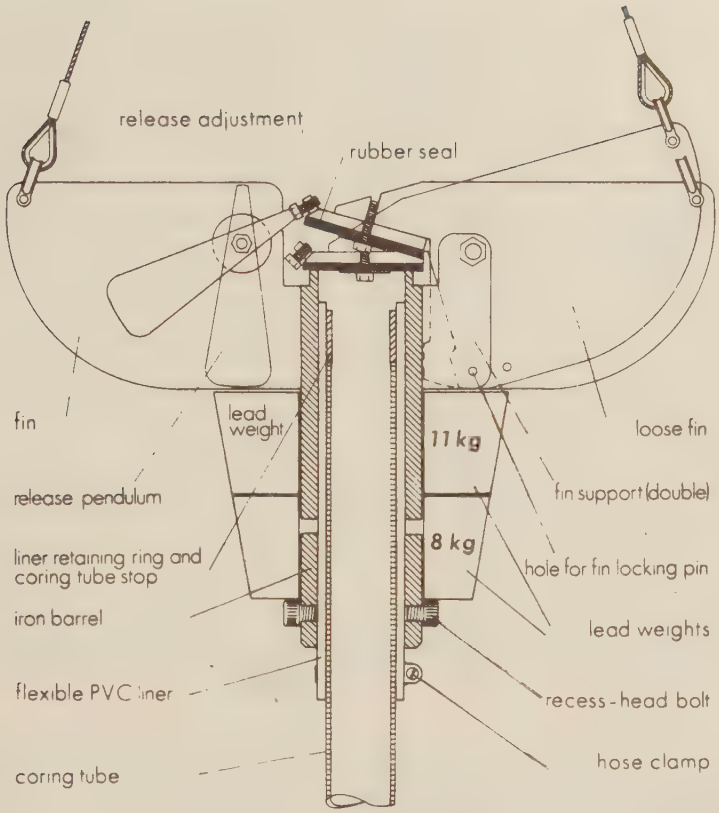


Figure 26. Side view and partial cross section of the high momentum corer. **Technical data:** weight adjustable from 10 to 30 kg; length 30 cm plus core tube; breadth 41 cm; 2 working parts. Valve: aperture 19.6 cm^2; closing force = hoisting force. Core tube: transparent acrylic glass, outer diameter 40 mm, inner diameter 36 or 34 mm; inner area 10.2 or 9.1 cm respectively
(From Meischner and Rumohr, 1974, with kind permission of Senckenbergische Naturforschende Gesellschaft, Frankfurt. © 1974).

Granulometric information is most important and for this the particle size analysis is made later on dried samples. Since the analysis and interpretation of results are quite complex, Holme and McIntyre (1971) or an appropriate marine sedimentological handbook should be consulted for details. The same sources are recommended also for instruction on geochemical analysis of sediments, which are important for ecological studies such as organic matter, carbon, nitrogen, phosphorus and carbonate content.

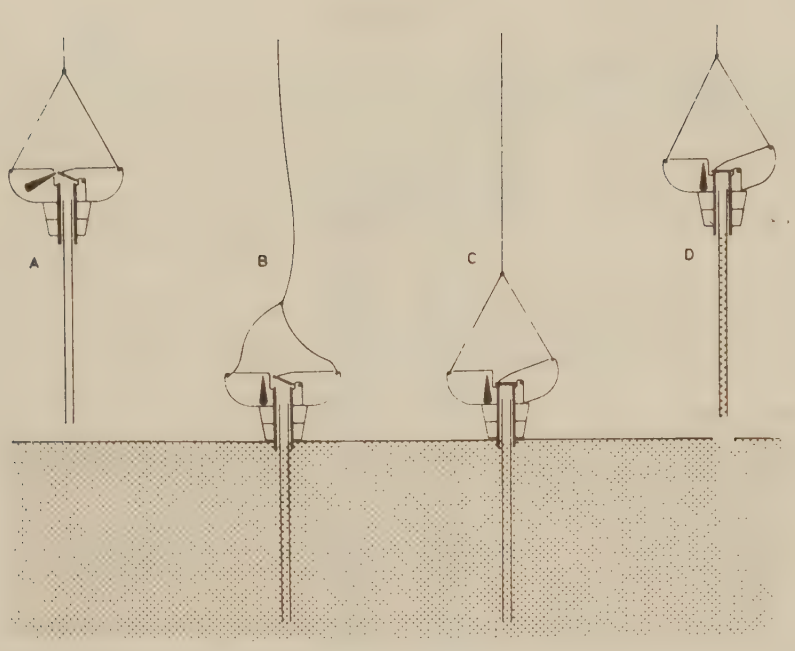


Figure 27. Typical phases in high momentum coring (schematic)
A - free fall shortly before contacting the sediment. Note that the valve is opened by the release pendulum; **B** - end of penetration, rope slack, flap valve open; **C** -start of hoisting, flap valve closed by taut rope prior to extraction of the core; **D** - hoisting, flap valve closed
(from Meischner and Rumohr, 1974, with kind permission of Senckenbergische Naturforschende Gesellschaft, Frankfurt. © 1974)

3. ASSESSMENT OF POLLUTION-INDUCED ECOSYSTEM MODIFICATIONS BY PELAGIC INVESTIGATIONS

The pelagic environment is the primary recipient and target for the great majority of pollutants and relevant stresses. This is also true for pelagic organisms, especially planktonic forms, for a number of reasons. The majority of planktonic organisms are small in size and volume, but the body surface is usually very large due to evolutionary adaptations to the pelagic life. This large relative surface makes them an optimal absorber of dissolved pollutants, and most probably also an adsorber. On the other hand, planktonic organisms have a short biological cycle and high metabolic activity; so they respond to pollution stresses with modified survival, growth and reproduction more quickly and significantly than the benthic or nektonic organisms (Pērès, 1976). This statement refers most likely to any kind of pollution stresses, yet quite clearly it can be illustrated only by the primary stage of eutrophication caused by sewage: increased nutrient level promotes and "explosive" growth of pelagic algae, although increased standing crops of some benthic algae are also part of the overall modification (see chapter 2 of this manual).

There is quite extensive scientific information on significant, easily measurable modifications of pelagic environments induced by pollutants and particularly for the Mediterranean area (Peres, 1976; Stirn, 1971). However, as a rule, these two authors refer to semi-enclosed bays, estuarine areas or lagoons (for example Athens, Marseille, North Adriatic, Lake of Tunis). The situation in open coastal sea areas seems to be entirely different. Dilution, dispersion, adsorptive settling and transportation by various and variable dynamic processes in the pelagic environment make it difficult to predict distributions of both pollutants and affected planktonic organisms. Most populations of planktonic organisms appear with significantly variable seasonal dynamics and successions whose natural factors are in general poorly understood, and much less is known about relevant pollution-induced modifications. Therefore, it is extremely hard to distinguish between naturally-occurring and pollution-induced phenomena. In addition, the known patch-distributions of planktonic organisms in general, and unpredictable "distribution disorders" in coastal zones in particular, present great obstacles to obtaining a suitable sample for pollution studies aimed at eventual decision-making. For these reasons and on the basis of practical experience, the assessment of pollution-induced ecosystem modifications made only by pelagic investigations is not often successful. However, in combination with benthic investigations and adequate environmental measurements, the pelagic investigations provide information of crucial importance. Even purely benthic approaches cannot be complete without the basic information on pelagic productivity in the sense of essential trophic potential.

3.1 Basic Terminology

Pelagic environment and communities are considered here as that part of a marine ecosystem which occupies only free waters (although being in one way or another dependent on benthic communities).

From the standpoint of vertical distribution the pelagic communities can be distinguished as epipelagic, inhabiting the trophogenic, photic upper layers; mesopelagic, diurnally migrating assemblages of intermediate layers, and bathypelagic or deeper communities which are no longer directly in touch with trophogenic layers. All pelagic communities are composed by nektonic, i.e. large, fast-moving organisms (fish, shrimps, squid, etc.) and planktonic organisms. For the purpose of this manual only planktonic, and mainly epipelagic, organisms shall be considered, although in particular cases and for shelfless areas of the Mediterranean, nektonic and mesopelagic communities should not be neglected in pollution-oriented investigations.

The autotrophic component of the plankton in the above sense is represented by the phytoplankton. For historical and practical reasons phytoplankton is graded by its size into nanoplankton (<20 µ) and phytomicroplankton (20 to 200 µ).

The zooplankton is constituted by, besides microbes, the heterotrophic component of the plankton, being composed of almost all major groups of animals, either as holoplanktonic organisms whose entire life-cycle is a pelagic one, or as meroplanktonic larvae of benthic organisms and as eggs and larvae of both demersal and pelagic fish (ichthyoplankton). For convenience, zooplankton can be graded into the following size groups:

- macroplankton: > 2 cm
- mesoplankton: 0.2 - 20 mm
- microplankton: 20 - 200 µ

In practice, however, macroplankton is considered as the whole fraction retained by a net with 250-µ mesh aperture or more, while microplankton is retained by nets with the finest mesh (53 -70 µ).

3.2 Principal Pollution-induced Modificatons of Pelagic Ecosystems

Ecosystem modifications caused by pollution stresses on pelagic environments can be summarized into the following categories, which correspond also to different methodologies:

(i) physical and chemical conditions and constituents of pelagic environments;

(ii) functional productivity, metabolism and energy circuits of the whole ecosystem and/or of its elements;

(iii) seasonal dynamics of standing crops for a community as a whole, its particular trophic levels and/or populations of dominant species;

(iv) composition and structure of communities.

Although knowledge of environmental conditions is essential in investigations recommended in this manual, the relevant methods are not considered in detail for they can be easily obtained from other sources. The functional approach which could produce an optimal understanding of pollution-induced ecosystem modifications is not considered in this manual at all as it is unfeasible. The recommendations in this manual are therefore focussed mainly on the investigations of communities by the relevant approaches.

3.3 Types of Planktonic Biota, Taxonomic Identification and Interpretation of Results

From a taxonomical point of view almost all classes of lower plants and invertebrates are present in pelagic communities, at least as larval stages. Therefore, the investigations of planktonic communities require skilled taxonomists either as members of the research team or as consultant specialists (see also section 2.2 above). The major taxonomic groups usually encountered in Mediterranean plankton are listed in Table IV with indications as to the specialists required for minimum information on the structure and basic trophic relationships in planktonic communities.

The analyses of plankton communities are no less time-consuming and expensive than for benthos, and the amount of useful information produced may not be worth the effort. Therefore, for most pollution-oriented projects a rather limited planktological team composed of at least the following professionals can be recommended:

- phytoplanktologist for taxonomic identifications of diatoms, coccolithophorids and common species of other major groups of phytoplanktonic algae;

- zooplanktologist for taxonomic identification of copepods and of common species of other pelagic crustaceans;

- zooplanktologist for biomass determinations and identification of common species of other crucially important pelagic groups (cnidarians, molluscs, chaetognates).

For efficient taxonomic analyses the following are, of course, essential: to-date taxonomic literature, reference collections, compilation of existing data on pelagic biota of the area, and the collaboration of external specialists for identification of those taxonomic groups not covered by the responsible research team.

As to the handling and interpretation of data obtained by quantitative taxonomic analysis, generally the same approaches as described for benthic investigations (see 2.2) can be applied, but specific features of pelagic communities should be pointed out:

(i) Due to problems of statistically adequate sampling of pelagic communities, the application of diversity indices and other measurements of community structure should be used most carefully;

(ii) Since the seasonal dynamics (standing crops, their overall trophic dispositions, species compositions and successions) are usually of far greater importance in pelagic than in benthic communities, the modifications induced by pollution effects might also be quite significant and measurable. Therefore this information is important. It has

Table IV

Specialists required for the identification of major Mediterrranean plankton groups

Taxonomic group	Specialist needed as:		Considered as a whole group
	Staff member	Temporary assistance	
PHYTOPLANKTON			
Bacillariophyceae	x		
Coccolithophoridae	x		
Dinoflagellata	(x)	x	
"Microflagellates"			x
ZOOPLANKTON			
Protozoa	(x)	x	
Hydromedusae		x	x
Sinophora	(x)	x	
Scyphozoa	x		
Ctenophora	(x)	x	
Mollusca (holoplanktonic)	(x)	x	
Mollusca (meroplanktonic)			x
Polychaeta (holoplanktonic)	(x)	x	
Polychaeta (meroplanktonic)			x
Cladocera	x		
Ostracoda	(x)	x	
Copepoda	x		
Mysidacea	(x)	x	
Amphipoda	x		
Euphausiacea	(x)	x	
Decapoda (meroplanktonic)			x
Chaetognatha	x		
Thaliacea	x		
Appendicularia	(x)	x	
Echinodermata (meroplanktonic)			x
Other invertebrate larvae			x
Ichthyoplankton			x

been shown in practice that these approaches usually provide more valuable information than diversity indices or similar structural measurements.

3.4 Basic Strategy of Pelagic Investigations and Design of Sampling Programmes

Most of the principles and methods for adequate strategy of pelagic investigations and sampling design are the same as those for benthic communities (see 2.3 and 2.4 above). However, due to obvious differences between benthic and pelagic environments and their biota, the following specific environmental data are also of crucial importance for the understanding and rational interpretation of pelagic community investigations:

(a) spatial and temporal distribution of characteristic water masses, their dynamic transport and relevant long-term fluctuations, based on measurements of salinity, temperature (density), dissolved oxygen and specific alkalinity, vertical thermohaline structure and stability of water masses;

(b) radiant energy and its vertical distribution within the euphotic layers for typical seasons;

(c) turbidity and colour of water masses as interrelated with underwater fate of radiant energy as well as indicating amounts and distribution of suspended solids of biotic, terrestrial and pollution origin;

(d) temporal and spatial distribution of macronutrients as total phosphorus, reactive phosphate, nitrates, nitrites, ammonia, reactive silicate and facultatively dissolved organic carbon and nitrogen.

A grid of sampling stations for environmental measurement it set up in accordance with previous knowledge on distribution of characteristic water masses, gradients of environmental or pollution-induced variations, etc. described in 2.3 above. At an individual station in situ measurable parameters such as temperature, salinity, oxygen and light are measured as continual vertical distribution; others are measured and water samples are taken at least in the following layers: at subsurface, above and below the thermocline, in compensation depth and in near-bottom layers. As a minimum, a monthly frequency of measurements and sampling is required, but during ecologically important transition periods (winter-spring, spring-summer, summer-autumn) more frequent observations are strongly recommended. For information on field and laboratory methods for environmental measurements see FAO (1975); Grasshoff (1976); Laevastu (1965); Strickland and Parsons (1968) and Schlieper (1972).

3.5 Sampling Design for Investigations of Pelagic Biota

Planktonic samples should ideally be taken at all stations designed for environmental measurements and at all depths as suggested above for each station. Unfortunately, such approaches are not feasible. Hydrographic conditions in coastal marine environments where pollution-focussed investigations are required are usually quite complex. Therefore, even the minimum number of stations needed for environmental measurements might be quite large, 20 to 30 stations at least. Environmental measurements and relevant chemical analysis for so many stations can be performed within a reasonable time, as can the phytoplanktonic and zooplanktonic biomass determination; however, quantitative taxonomic analyses of pelagic communities would present a very difficult and time-consuming task, which may not be worthwhile. Therefore, the following restricted sampling design is suggested:

(a) Within the area of each distinct water mass (such as offshore, coastal estuarine-influenced) two stations are selected, one in the centre and the other close to the transition into a neighbouring water mass;

(b) similarly, 2 to 5 stations are located along the radius of environmental gradient areas if present (such as river mouth, large pollution sources);

(c) besides stations designed within likely pollution area, a number of stations should be sampled within a near, environmentally comparable but pollution-free area (for the purpose of parallel investigations;

(d) stations designed for plankton sampling and for environmental measurements should always overlap.

SUMMARY

As it is extremely hard to distinguish between naturally-occurring and pollution-induced changes in the structure of pelagic communities and their productivity, and assessment of pollution-induced ecosystem modifications is rather difficult but, in combination with benthic and environmental investigations, pelagic investigations provide information of crucial importance.

The basis of both pelagic sampling design and actual investigations is a solid knowledge of relevant environmental conditions in space and time, particularly hydrography of water masses, radiant energy, optical conditions, nutrients, etc. The methodology for relevant environmental measurement is obtainable from standard manuals (see 3.5).

For the sampling design of pelagic investigations, an approach similar to the one described for benthos (see 2.3) is recommended, i.e. a minimum of two stations within each area of distinctly different water masses and a higher number of stations along radials of environmental pollution gradients and in transition zones. Surface, thermocline and near bottom layers should be sampled at each station with at least a monghly frequently the year round, and a bi-weekly frequency during blooming periods.

3.6 Sampling and Analysis of Phytoplankton [2/]

3.6.1 Sampling of phytoplankton

The most satisfactory and simple devices for obtaining phytoplankton samples are the closing water-bottles actuated by messengers. They should be made entirely of non-toxic plastic and partly rubber materials. Van Dorn and similar types of large volume (3 to 5 litre-) samplers are quite suitable and inexpensive, and can also be home-made. Standard or even plastic coated Nansen bottles should not be used for phytoplankton sampling.

One large-volume sample is taken at each pre-selected depth of a given station. The subsurface sample (upper sampler opening should be 20 cm below surface) must also be taken by a sampler, never by a bucket. The water is emptied from the sampler into a carefully cleaned glass container (Pyrex or similar quality), mixed by a glass rod and divided into biomass and phytoplankton subsamples. Biomass determination requires a volume of 1 litre for more productive conditions or even less for "blooming" conditions, and up to 3 litres for oligotrophic waters, such as typically occur in the Mediterranean Sea. This means that one sample collected by a standard 5-litre van Dorn sampler is sufficient for both biomass and phytoplankton subsamples, and there is also enough water left for basic chemical analyses if needed.

3.6.2 Storage, fixation and preservation of samples

Biomass determinations can be performed only with live samples. If possible, at least the filtration step of biomass determination is done on the spot immediately after the samples have

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[2/] For advanced information see Unesco Phytoplankton Manual (Sournia, 1978)

been obtained, otherwise they should be kept (in carefully cleaned 3-litre plastic bottles) in a dark, cool place or refrigerator (8-10°C) until they reach a laboratory. In any case, determination should start at the latest 24 h after the sampling.

Phytoplankton analyses are made routinely on preserved samples. An aliquot of fresh sample (500 - 1000 ml) is placed in a clean, dark glass bottle or non-transparent plastic container, fixated and carefully labelled (with a label also inside the bottle). Although Lugol's solution[3/] is quite commonly used for phytoplankton, weak neutral formalin fixation is recommended (1.5 percent formalin, i.e. 0.6 percent formaldehyde). Formalin is most conveniently buffered by the addition of hexamine (hexamethylene-tetramine) at a rate which makes the final pH of samples about 8 (7.8-8.2) in order to prevent dissolution of phytoplankters' skeletons (coccolipthophorids), which are crucially important for taxonomic identifications. To ensure good mixing, stock formalin should be put into the sample bottle first (e.g. 8 ml of 38 percent formaldehyde per 500 ml of sample) and then the sample added. Formalin-fixated samples can be stored in a dark place at room temperature for an unlimited time, but the pH of samples should be checked occasionally for the above mentioned reason. However, it is advisable to work on samples as soon as possible since, in spite of all precautions, skeletons become modified. Since all types of standard fixatives distort or destroy delicate naked flagellates (a particularly important element in coastal or polluted waters) they can only be studied alive. For this purpose usually only small aliquots (100 ml) are needed and they can be taken from the live biomass samples upon arrival in the laboratory or subsampled and kept as mentioned above.

3.6.3 Determination of total phytoplankton biomass

In principle, a number of methods can be used for the determination of total phytoplankton standing crops by measurements of biomass such as dry weight, carbon, nitrogen and ATP content of phytoplankton concentrated from a given volume of a water sample by centrifugation or filtration. The essential disadvantage common to all these methods is that the determination includes detritic, bacterial and some microzooplanktonic elements besides the phytoplankton.

An indirect estimation of dry weight or carbon biomass can be obtained also from phytoplankton cellular volumes by applying conversion factors obtained either from literature or experimentally. For this purpose the number of individuals of each species as obtained from counts of phytoplankton samples (see 3.6.4 below) must be multiplied by the average cell volume calculated from dimensions of cells, assuming that the form corresponds roughly to simple geometrical bodies. Although this method is time-consuming and provides very rough data the advantage is that it gives information on how the biomass is shared by dominant phytoplanktonic species. Therefore, it can be recommended especially for research projects focussed on the investigation of functional productivity and energy circuits. The usual estimation of phytoplanktonic biomass is, however, the chlorophyll content of total standing crop per unit of sea water volume (usually in µg/l) and as such can be also recommended within the scope of this manual. Since the relevant methods are described in detail in easily available handbooks (SCOR-Unesco, 1966; Strickland and Parsons, 1968; Vollenweider, 1974) only an outline of the analytical method and the filtration technique are described here.

Phytoplankton from seawater samples is concentrated by filtering through cellulose of cellulose-derivative membrane filters of 0.45 - 0.65 µ pore size[4/]. Before filtration, the filters should be covered with a layer of about 10 mg/cm^2 of finely powdered $MgCO_3$ serving as a prefilter and as a precaution against acidity and consequent degradation of pigments. For this purpose $MgCO_3$ is suspended in distilled water and a suitable amount (e.g. 20 ml of stock suspension = 6.5 g $MgCO_3$ per 1000 ml distilled water) is filtered until dry. For this and subsequent filtration of the sample the filter is held clamped in a special funnel, lying on the base made of sintered glass or porous

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[3/] 2 g potassium iodate and 1 g iodine in 200 ml distilled water. The solution is added to the sample in amounts which turn the sample the colour of weak tea.

[4/] Millipore HA type 47-mm diameter filters are most commonly used. Fibreglass filters, although they are cheaper and filter more rapidly, are not recommended because of large and unevenly sized porosity.

plastic plate, which is connected to a vacuum source. The suction pressure during the filtration should not exceed 0.3 kp cm^{-2} in order to avoid losses due to possible fragmentation of delicate phytoplankters. The assembled filtering unit is shown in Figure 28; it can be purchased from the same suppliers as the membrane filters.

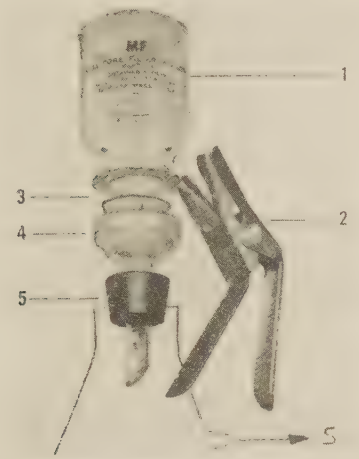

Figure 28. Membrane filtration unit: combined funnel, filter clamp and suction bottle
(From Millipore Corporation, 1976, with kind permission of Millipore Corporation, Bedford, MA. © 1976)

The chlorophyll determination should proceed immediately after the filtration is completed, but exceptionally filters can be stored for a short time (maximum 24 h) in a desiccator at low temperature (4°C) or in a deep-freezer. The whole filter with the $MgCO_3$ and phytoplankton filtrate is placed in a test tube which fits a teflon pestle (such as tissue grinders). A volume of extraction solvent[5/] (e.g. 3 ml) is added and then the filter is ground for 1 min at 500 rpm. More solvent is added (e.g. 7 ml), the contents mixed and left for at least 10 min extraction time. The whole extract is cleared by centrifugation (10 min at 5000 g) and is then ready for spectrophotometric estimation of chlorophyll concentration. The trichromatic method suggested by SCOR-Unesco (1966) is recommended. A good spetrophotometer which fits cuvettes of up to 10 cm path length is a prerequisite for reliable determination, especially for such low chlorophyll concentrations as are usually encountered in the Mediterranean Sea.

Due to the known fluorescence of chlorophyll, a number of relevant fluorometric methods, e.g. extractive and by measurements in vivo, have been developed which have some advantage in comparison with the method described above, i.e. high sensitivity and small sample volume required even for in situ measurements. They are rarely applied by Mediterranean laboratories and hence results might be less comparable.

3.6.4 Quantitative taxonomic analysis of phytoplankton

Quantitative analysis of phytoplankton provides taxonomic identification on possibly all species present in a sample (except problematic naked flagellates), on the total phytoplankton density (total abundance), as well as on the enumeration of abundance for each dominant and common species, i.e. arbitrarily those species whose cumulative abundance constitute about 80 percent of the total abundance in a sample[6/].

Microscopic examination, needed both for counting and identification of species, cannot be done by the observation of a "drop-subsample" taken directly from the original sample since phytoplankton densities, particularly in oligotrophic Mediterranean waters, are much too low for such an approach

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5/ Recommended solvent is 90 percent acetone p.a.

6/ Total and specific abundances are usually expressed as number of cells/litre. Colonial forms are also counted by number, not as colonies, except for (rarely occurring) filamentous cyanophytes.

(usually 10^4 -10^5 cells/l). Exceptions are occasionally occurring extreme "blooms", with densities of 5 - 20 x 10^6 cells/l. Therefore, prior to all abundance estimations, some kind of sample preconcentration is required and this can be done by the following techniques.

3.6.4.1 Centrifugation of samples

Centrifugation has a number of disadvantages and is not advisable for routine work. However, for observations of live samples needed for fixation-sensitive naked flagellates, it is still the best method although losses may reach 30 to 50 percent of the organisms actually present in a sample. The minimum speed of centrifugation is 10 000 rpm for 5 to 10 minutes. After centrifugation, the supernatant is completely removed and the settled phytoplankton mixed with a constant volume of clean sea water (e.g. 2 ml) from which a number of drop-subsamples are examined and counted on a haemacytometer under a regular research microscope equipped with phase contrast optics. For the calculation of phytoplankton abundance, specific characteristics of a haemacytometer and preconcentration ratios provide the appropriate factors. For example, 100 ml original sample was concentrated to 2 ml, of which 3.2 mm was counted (haemacytometer field volume = 4 mm x 4 mm x 0.2 mm), giving a total of n cells. The total of N cells/l is obtained by the following calculation:

$$N = n \frac{2000\ mm^2}{3 \cdot 2\ mm^2} \cdot 10 = n \cdot 6250 \quad [cells/l]$$

3.6.4.2 Membrane filtration

Membrane filtration (described in 3.7.3 above) offers a convenient technique for quantitative analyses 7/, but it can be recommended only tentatively for routine procedures in laboratories which cannot afford Utermohl equipment (see 3.6.4.3).

Fixed-preserved phytoplankton, or live samples if required (500 to 1000 ml or more) are passed through a membrane filter in one lot. Before the end of filtration, the walls of the receiving funnel are rinsed in clean sea water (membrane-filtered). Examination of the filter-concentrated phytoplankton can be made by two different approaches:

(i) "Off-filter" examination - Filtration is stopped just before the filter is quite dry. The filter is removed and placed with its margin on the edge of a watch glass. Using a very fine brush and microjet sprays of filtered fixative 8/, the phytoplankton is flushed from filter surface as completely as possible into the watch glass and then into a small vial. The final volume of concentrated phytoplankton must, for obvious reasons, be rather small (5 ml) (which requires some practical experience); if not, the sample must be reconcentrated by centrifugation. By this method, an experienced researcher can obtain quantitative concentrations with less than 30 percent losses of the actual phytoplankton density; however, it is most advisable to check individual efficiency by the processing of monoalgal suspensions whose density was determined by direct counts of drop-subsamples. The concentrated phytoplankton samples thus obtained are examined and counted in haemacytometers as explained in 3.6.4.1.

(ii) "On-filter" examination - After sample filtration has been completed, the filter remains clamped in the funnel, it is washed with increasingly diluted filtered sea water (75, 50, 25

7/ Note: For this purpose membrane filters should not be covered by MgCO layer or prefilters

8/ 1.5 percent formalin in membrane-filtered sea water

and 10 percent) and finally with distilled water made basic (pH 7.5) by the addition of NH_4OH. The filter and material are then dried by successive washing with 10, 30, 50, 75 and 95 percent ethanol solutions (15 ml each) and stained. For this purpose the filter disk is covered by a layer (2 mm) of 0.1 percent solution of fast green pigment in 95 percent ethanol solution and allowed to stand for about 20 minutes. The stain is passed through the filter, which is rinsed with 20 ml pure ethanol. The filter is removed, its edges trimmed, and it is placed for 10 min, filtering surface up, with a few drops of immersion oil (clearing agent) on a regular microscope slide. Then the filter is placed on another slide with a few drops of xylene balsam, and a few drops of balsam are also added to the upper surface of the cleared filter, which is permanently covered by thin cover glass (No. 1). After drying (at 40°C), the preparation is ready for examination. Provided that the entire surface of the filter was examined and all phytoplankters counted, the data obtained are absolute and refer to the volume of a filtered sample; hence no calculations are needed. In practice, however, less abundant species are counted for the whole surface and dominants from fractions (transects). In the latter case the counts are multiplied by the appropriate factor, i.e. ratio between total and examined filter surface. (For details of this method see Holmes, 1962; de Noyelles, 1968).

3.6.4.3 Settling techniques

Although the settling technique can be applied in various ways, e.g. settled phytoplankton removal from the bottom of large (1 to 5-l) settling cylinders for examination with regular microscopes, only the method described by Utermöhl (1958), which is efficient and widely used, is mentioned here. The whole set of items needed for this techniques can be purchased from a supplier which sells the basic instrument, i.e. inverted microscope. The microscope should be of high quality and equipped with phase contrast optics. This instrument differs from the conventional microscope in that the objectives are mounted below the stage and the illumination comes from above, hence it allows cylindrical settling - counting chambers with thin glass bottoms (cover slide No. 1½) to be placed on the stage and sedimented phytoplankton to be examined from below. Single chambers for settling 1-, 5-, 10-, 25- and 50- ml subsamples and combined chambers for subsamples of 10, 25, 50 and 100 ml are available.

Due to usually low phytoplankton densities in Mediterranean oligotrophic waters, combined chambers are most often needed so it is advisable to have a number of them (4 to 6) for serial examinations. This chamber consists of two parts, a bottom plate chamber and a chamber cylinder of 50- or 100-ml volume. After a suitable time given for the phytoplankton to settle on the bottom plate, the chamber cylinder is removed transversely and only the bottom plate chamber placed on the microscope and examined.

According to the expected density of phytoplankton from the original preserved sample (prior to subsampling it should be throroughly agitated) a subsample is poured into a single chamber (25 or 10 ml for eutrophic waters) or into a 100-ml combined chamber for settling. Since some nanoplankters sink quite slowly (3 mm/hour) settling-sedimentation time in hours must be at least three times the height of the sedimentation chamber in centimetres. In order to avoid the formation of bubbles on the cylinder walls the temperature of the original sample should be about the same as the room temperature where the chambers are placed for sedimentation.

Microscopic examination and counting of phytoplankters settled on the bottom is carried out in two stages: first, the whole bottom area is scanned under a low magnification for large, usually scarce species, then nanoplanktonic and dominant species in the fields of at least two crossed diameter transects are examined and counted using a high power objective. The total abundances of species in a subsample are found by multiplying the number of cells counted in the transects by the ratio of the whole chamber area to the area of the examined transects.

In order to avoid errors that may arise from low or overcrowded densities of phytoplankters the counting of several subsamples in chambers of different sizes is recommended. Generally, at least 100 individuals of every important species should be counted, possibly at different magnifications because smaller cells may be overlooked at low magnifications or detritic particles counted for nanoplankters. It happens that, for the purpose of problematic taxonomic identifications, settled phytoplankton has to be removed from the chamber (e.g. in order to send material to a specialist); this can be done by capillary pipettes or a special technique described by Haller Nielsen (1950). Regardless of the accuracy level of counting procedures, the statistics of subsample, sample and sampling variables should be respected, and directly obtained data verified by appropriate methods. For instructions and further references see Vollenweider (1974).

SUMMARY

The following procedures for phytoplankton investigations are recommended:

(a) Samples of seawater are obtained by van Dorn or similar large-size atoxic samplers. Within the subarea of a station, a number of samples is taken at random, mixed into a large composite sample from which mixed subsamples are taken for phytoplankton, chlorophyll and chemical analyses. The last two operations must be done on fresh samples (or deep-frozen for short periods). A phytoplankton sample of a volume of at least 1 litre can be preserved with the addition of neutralized formalin up to a final concentration of 1.5-2 percent. It is recommended that phytoplankton samples be examined in order to make observations on naked flagellates, the identification and counting of which in a preserved state is almost impossible.

(b) Phytoplankton community analysis is made by the taxonomic identification of, at least, dominant species of diatoms, coccolithophorids, dinoflagellates and naked flagellates, and by the enumeration of specific group and total densities. The method suggested is the Utermõhl settling and inverted microscope method; however, similar results can be obtained by membrane filtration or high-speed centrifugation (see 3.6.4.1 and 3.6.4.2).

(c) The phytoplankton biomass can be determined indirectly by calculating volumes of cells, on the basis of the above-mentioned data or by sophisticated direct analyses; however, for routine estimations, chlorophyll determinations by the SCOR/Unesco trichromatic or by fluometric methods are recommended (see 3.6.3).

3.7. Sampling and Analyses of Zooplankton

There is quite an important difference in structure, size and distribution between microzooplanktonic and meso- and macrozooplanktonic communities. Consequently, the sampling and examination methods cannot be the same for both; they are considered here separately, but this does not mean that one approach excludes the other for purposes of pollution-oriented ecosystem investigations. Information on both micro- and mesozooplankton is probably equally important, although the former is usually neglected. This is understandable from historial and technical viewpoints but it is scientifically wrong since even microzooplankters, such as neritic protozoans, most likely represent a crucially important trophic link, particularly in polluted environments.

3.7.1 Sampling, preservation and examination of microzooplankton

Although some microzooplanktologists prefer fine-mesh (53 to 75 µ) nets for microzooplankton sampling, for regular sampling closing water-samplers are recommended. Large volume (10 l) van Dorn, Niskin and similar types of samplers have been found the most suitable. The 5-l van Dorn sampler can also be used, provided that the contents of at least two samples taken successively at the same depth at the same station are integrated into one sample.

Immediately after the sample is obtained it should be filtered through a 20-μ sieve, or at least through the finest mesh (40 μ to 53 μ). The filtrate is washed into a glass container with 190 ml of filtered sea water and 10 ml concentrated formaline (38-40% formaldehyde) added. The pH of this solution is then adjusted at 8.0 to 8.2 by the addition of borax ($Na_2B_4O_7 \cdot 10\ H_2O$) in amounts determined empirically (by tests made on sample-free solutions). Special investigations on non-loricate ciliates require subsamples preserved in a mixture of equal parts of 80 percent ethanol and 4 percent formalin seawater solution, live samples or fixation with osmic acid.

Examination and counting is done with an inverted microscope by a similar procedure to that described for phytoplankton (see 3.6.4.3). First, a 50-ml subsample (taken after the original sample has been turned upside-down for a minute) is poured into a combined chamber and left to settle overnight. In order to obtain reliable information, the counting of the subsample should include about 100 specimens for every dominant species; if not, the rest of the original sample (150 ml) must be re-concentrated by being filtered through a 20-μ sieve and washed into a 50-ml chamber, settled and examined again.

Measurement of microzooplankton biomass presents a difficult task; however, it may be estimated from the calculation of the volume (see Dybern et al., 1976).

3.7.2 Sampling, preservation and examination of mesozooplankton

Although pumping and other sophisticated techniques are sometimes used for zooplankton sampling, the universal gear remains a plankton net. This consists essentially of a cone of bolting silk or equivalent synthetic tissue fixed on a metal ring. The ring is connected to a towing warp. The opening and end parts of the net are reinforced by thin canvas or similar material. A suitable metal or plastic bucket (volume 200-1000 ml) is fixed to the end of the net in such a way as to be easily detachable; it is equipped with longitudinal windows covered by the same material as the net. The opening ring and net end are connected with ropes which hold the weight and towing force, hence protecting delicate parts of the net.

There are many types of plankton net in common use and many are applied for specific purposes. Although in the Mediterranean Juday-Bogorov and Hensen type nets are largely accepted in utilized by Mediterranean planktologists (see Tranter, 1968; Magazzu, 1978), for the purpose of this manual the WP-2 net is recommended. This is actually a modification of the Nansen closing net shown in Figure 29. The net is equipped with a Nansen system which allows the noose to be laced and the net closed at the desired depth. This system is triggered from the surface by a messenger which falls on the releasing element, shown in Figure 30. Recommended net material is nylon Nytal 7 P with a 200-μ mesh. The bucket has a volume of 200-ml, is 7.5 cm in diameter, is made of PVC or light brass, with windows covered by the same material as the net. The end of the net should fit flush into the cod-end so that there is no pocket in which plankton can collect. The 25-kg lead weight is attached to eyelets on the cold-end in such a way that supporting longitudinal ropes take the force, not the filtering material of the net.

For the purpose of this manual a double stratified zooplankton sampling is recommended. It means that at each selected station two layers are sampled:

(i) the shallow epipelagic zone corresponding to the average summer mixed layer, i.e. from the average thermocline depth (usually 30 to 40 m) to the surface;

(ii) the deep epipelagic zone, i.e. from the sea bottom to the thermocline, except if the depth exceeds 200 m (shelf limit), in which case sampling is carried out from 200 m to the above layer (30 to 40 m).

Throughout the annual cycle the same layers are sampled regardless of whether the thermic stratification is developed or not. Each layer is sampled twice in order to obtain one sample for biomass determination and another for quantitative taxonomic analysis of the zooplankton community.

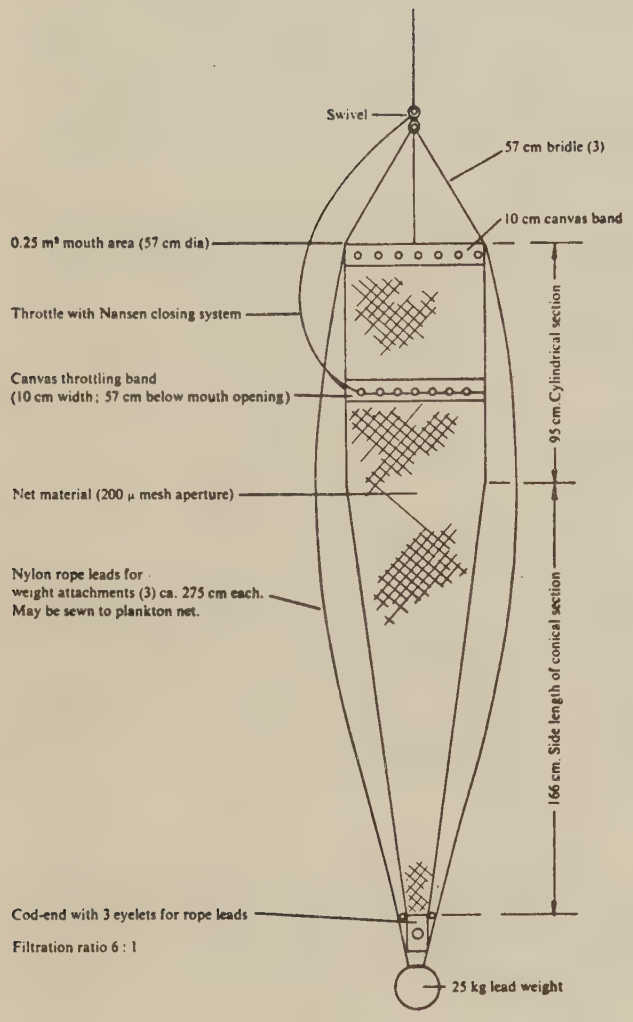
Swivel
57 cm bridle (3)
10 cm canvas band
0.25 m² mouth area (57 cm dia)
Throttle with Nansen closing system
Canvas throttling band
(10 cm width; 57 cm below mouth opening)
Net material (200 μ mesh aperture)
Nylon rope leads for
weight attachments (3) ca. 275 cm each.
May be sewn to plankton net.
Cod-end with 3 eyelets for rope leads
Filtration ratio 6 : 1
25 kg lead weight
95 cm. Cylindrical section
166 cm. Side length of conical section

Figure 29. Mesozooplankton net WP-2 (From Tranter, with kind permission of Unesco Press, Paris. © 1968)

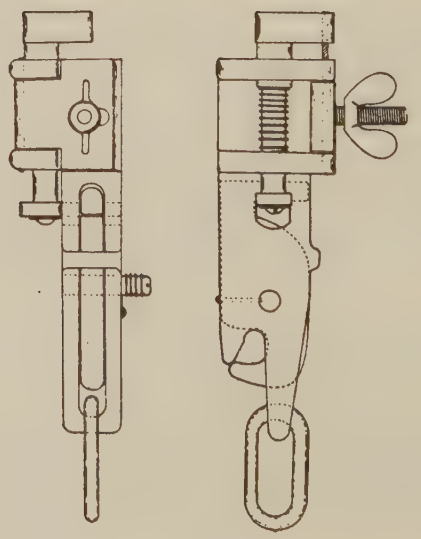

Figure 30. Triggering mechanisms for closing zooplankton nets (From Sverdrup et al., 1946, with kind permission of Prentice Hall Inc., Englewood Cliffs, N.J. © 1946, renewed 1970)

The sampling is done entirely by vertical hauls, with the same WP-2 type closing net [9]. Therefore, the samples will be comparable and also the volumes of water filtered. Providing that sampling conditions recommended below are about the same, the filtered volume can be calculated as the volume of a hauled cylinder, i.e. surface of the net mouth (0.25 m^2) multiplied by the length of a vertical haul (x m), reduced by 20 percent to allow for known losses due to physical and biotic avoidances. It is useful, although still not fully reliable, to measure filtered volume by a calibrated flowmeter placed half-way between the centre and the rim of the mouth (Tranter, 1968).

Vertical sampling tows are best made from a hydrographic platform on the side or from the stern of a research or fishing vessel. The plankton net is towed by a winch-handled hydrographic wire (4 to 6 mm diameter) and, if possible, over a crane or boom (see Figure 14). This allows the net to be hung above the deck facilitating washing and sample handling.

To obtain a sample, the net is lowered at a steady speed (maximum 1 m/sec) to the desired depth as determined by the reading on a meter-wheel or by meter-markers made on the wire. Ideally, the wire penetrates the water column vertically; if not, the wire angle is measured, the length of the wire needed to reach the desired depth trigonometrically estimated and corrected. The plankton net is hauled at 0.5 m/sec until it reaches the upper limit of a sampled layer (e.g. from 200 to 40 m) where it is closed by a messenger dropped from the surface along the wire on the releasing mechanism. The net is hauled to the deck and while hanging free all particles are carefully washed with sea water (preferably running sea water) from the filtering surfaces into the bucket. Then the bucket is released, emptied into a container and its walls and windows also carefully washed into it by jets of sea water from a plastic squeeze bottle.

The sample obtained, if meant for biomass determination, is immediately filtered until dry through a sieve with 200-μ mesh (acrylic glass cylinder 5 cm in height and 10 cm in diameter) and rinsed twice with distilled water for desalting. Concentrated plankton from the sieve is straightaway transferred (using jets of distilled water) into pre-weighted crucibles. For a short time (12 h) they can be stored in a refrigerator or deep-frozen until the start of biomass determinations. Biomass as dry weight and ash-free matter is determined by the methods described in 2.4.6 above, yet maximum temperatures of 70°C for drying and 480°C for ashing are recommended for zooplankton samples.

A sample determined for quantitative taxonomic analyses must be fixated immediately after being collected. Placed in a suitable container [10], it is diluted with sea water up to 3/4 of its volume and 38 to 40 percent buffered formaldehyde [11], added in amounts needed to make the final 2 percent formaldehyde concentration, i.e. 5 to 6 ml formalin/95 ml sea water. The sample is well mixed by gentle agitation and a label placed inside the container, which is also marked on the outside with the date, depth and station. After a week or so the pH of the formalin solution must be checked and, if it found too low, replaced by a new one. Also at that time the sample can be concentrated and transferred for final preservation.

The examination and counting of zooplankton samples is done in several steps. First, the whole sample is examined for large species (>10 mm) which are all sorted out and identified. It is advisable, although time-consuming, to examine the whole sample also for scarce species (excluding copepods) which may not appear in a subsample. For this purpose successive portions of a sample are examined in a petri dish under a good dissecting microscope. The bulk of the sample cannot be examined as a hole,

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[9] After a certain period the filtering material of the net becomes distorted or clogged and should be replaced by a new material

[10] ½ to 1-litre glass jars with clamp-top lids with rubber washers ("Weck") are recommended for shipboard use, later when fixation is completed, samples can be concentrated and transferred to smaller plastic or glass containers (200 to 300 ml)

[11] About 2 g of borax/100 ml of 38 to 40 percent formaldehyde is added to raise the pH of formalin to about 8.2

at least not while doing serial work, and must therefore be subsampled. For this purpose the use of the Folsom plankton splitter (Fig. 31) is recommended; it can be purchased or made (ideally of acrylic glass). This device separates the whole sample into two equal portions (from the septum divided drum into both boxes). The half sample is diluted and returned into operation to obtain $\frac{1}{4}$ sample, and so on. For routine purposes, and if the sample is relatively rich, usually a 1/16 fraction subsample is sufficient. The subsample must also be examined in portions; suitable portions are pipetted into a counting tray (Fig. 31) or petri dish, the bottom of which has a 5-mm square grid (drawn by diamond) to facilitate examination. The counting of easily recognizable species and major taxonomic groups can be done straightaway, but other groups or other taxa are transferred from the bulk into separate vials for identification later by a specialist. Skilled and patient technicians must assist planktologists in this operation, leaving to them time needed for taxonomic identifications, compilation of quantitative data and interpretation of results.

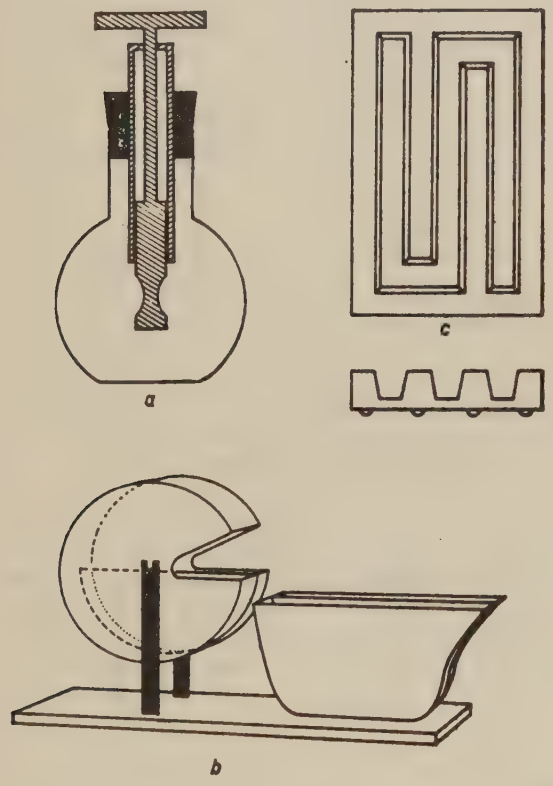

Figure 31. Some tools for subsampling of zooplankton samples: (a) stempel pipette; (b) Folsom splitter; (c) counting tray
(From Schlieper, 1972, with kind permission of Sidgwick and Jackson Ltd., London. © 1972).

SUMMARY

In order to obtain information on zooplanktonic communities and standing stocks relevant to pollution-oriented investigations, the following methods are recommended:

(a) Microzooplankton is collected most satisfactorily by large-size water samplers (10 litres); samples are immediately filtered through 20-μ mesh and preserved in 2 percent neutralized sea water formalin (= 0.8 percent formaldehyde). Identification and enumeration are done by a settling technique similar to that described above for phytoplankton (see 3.6.4).

(b) Mesozooplankton is collected by stratified vertical hauls with closing plankton net, type WP-2 200- mesh, as recommended by Tranter (1968); the filtered volume of the seawater column is calculated or measured by calibrated flowmeters. Samples are then quantitatively transferred into jars, carefully labelled and preserved in neutralized 5 percent seawater formalin (= 2 percent formaldehyde).

(c) Analysis of zooplanktonic community structure and determination of specific group and total abundances are done entirely by microscopic sorting and identification. Usually most zooplanktonic biota can be identified and counted at species level, except for mesoplankton and copepods; for the latter, the dominant species must be identified and quantitative data obtained. Large-size and scarce species are taken out of the whole samples for treatment; for the more abundant elements a suitable adequate subsampling approach can be applied.

(d) Although very approximative data on zooplankton biomass can be obtained from preserved samples, parallel samples should be collected for this purpose and kept deep-frozen until analysis. The procedure for the biomass determination is the same as described for benthos (see 2.4.4. above); however, samples should be carefully desalted and the whole procedure performed at a higher precision level.

4. REFERENCES

Ankar, S. and B.O. Jansson, 1973 Effects of an unusual natural temperature increase on a Baltic soft bottom community. Mar.Biol., 18:9-18

Avčin, A. et al., 1973 Comparison of benthic communities of the bays of Koper and Strunjan in relation to pollution-induced differences. Biol.Vestn., 22:171-208 (in Slovenian, Engl.summary)

Arrhenius, O., 1918 En studie över yta och arter. Sven.Bot.Tidskr., 12:180-8

Barnett, P.R.O. and B.L.S. Hardy, 1967 A diver operated quantitative sampler for sand macrofauna. Helgol.Wiss.Meeresunters., 15:390-8

Boudouresque, C.F., 1971 Methodes d'etude qualitative et quantitative du benthos. Tethys, 3:79-104

_____, 1974 Aire minima et peuplements algaux marins. Bull.Soc.Phycol.France, 19:141-57

Brandt, A. von, 1981 Fish catching methods of the world. West Byfleet, Surrey, Fishing News (Books), 240 p.

Briba, C. and J.P. Reys, 1966 Modification d'une benne "orange peel" pour les prélèvements quantitatifs du benthos de substrats meubles. Rec.Trav.Stn.Mar.Endoume, 21(57):117-21

de Noyelles, F. Jr., 1968 A strained-organism filter technique for concentrating phytoplankton. Limnol. Oceanogr., 13(3):562-5

Dybern, B.I., H. Ackefors and R. Elmgren (eds), 1976 Recommendations on methods for marine biological studies in the Baltic Sea. Baltic Mar.Biol.Publ., (1):98 p.

Edmondson, W.T. and G.G. Winberg (eds), 1971 A manual on methods for the assessment of secondary productivity in fresh waters. IBP Handb., (17):358 p.

FAO, 1975 Manual of methods in aquatic environment research. Part 1. Methods for detection, measurement and monitoring of water pollution. FAO Fish.Tech.Pap., (137):238 p.

Finnish IBP-PM Group, 1969 Quantitative sampling equipment for the littoral benthos. Int.Rev.Gesamt. Hydrobiol., 54(2):185-93

Gleason, H.A., 1922 On the relation between species and area. Ecology, 3:156-62

Grasshoff, K., 1976 Methods of seawater analysis. Weinheim, Verlag Chemie, 317 p.

Gray, J.S., 1971 Sample size and sample frequency in relation to the quantitative sampling of sand meiofauna. Smithsonian Contrib.Zool., 76:183-90

Haller Nielsen, P., 1950 An auxiliary apparatus for plankton studies by means of the sedimentation method. J.Cons.CIEM, 16(3):307-9

Hiscock, K. and R. Hoare, 1973 A portable suction sampler for rock epibiota. Helgol.Wiss.Meeresunters., 25:35-8

Holme, N.A., 1959 A hopper for use when sieving bottom samples at sea. J.Mar.Biol.Assoc.U.K., 38:525-9

Holme, N.A. and A.D. McIntyre (eds), 1971 Methods for the study of marine benthos. IBP Handb., (16):334 p.

Holmes, R.W., 1962 The preparation of marine phytoplankton for microscopic examination and enumeration of molecular filters. Spec.Sci.Rep.USFWS(Fish.), (433):9 p.

Hulings, C.N. and J.S. Gray, 1971 A manual for the study of meiofauna. Smithsonian Contrib.Zool., (78):84 p.

Jaccard, P., 1928 Die statistisch-floristische Methode als Grundlage der Pflanzensoziologie. Handbuch der biologischen Arbeitsmethoden, II/5, edited by E. Abderhalden. Berlin, Urban und Schwarzenberg, pp. 165-202

Jansson, A.M., 1974 Community structure, modelling and simulation of the Cladophora ecosystem in the Baltic Sea. Contrib.Askö Lab., (5):130 p.

Kangas, P., 1972 Quantitative sampling equipment for the littoral benthos. IBP i Norden, 9:9-16

Kautsky, N., 1974 Quantitative investigations of the red algae belt fauna in the Asko area, northern Baltic proper. Contrib.Askö Lab., (3):29 p.

Laevastu, T., 1965 Manual of methods in fishery biology. FAO Man.Fish.Sci., (1):10 fasc.

Lassig, J., 1956 An improvement to the van Veen bottom grab. J.Cons.CIEM, 29:352-3

Longhurst, A.R., 1959 The sampling problem in benthic ecology. Proc.N.Z.Ecol.Soc., 6:8-12

Magazzu, G., 1978 Metodi per lo studio del plancton e della produzione primaria. Milano, Edizioni GM, 100 p.

Margalef, R., 1968 Perspectives in ecological theory. Chicago, University of Chicago Press, 111 p.

Massé, H., 1970 La sucense hydraulique, bilan de quatre annees d'emploi, sa manipulation, ses avantages et inconvenients. Tethys, 2:547-56

Meischner, D. and J. Rumohr, A light-weight, high momentum gravity corer for subaqueous sediments.
1974 Senckenb.Marit., 6:105-16

Millipore Corporation, Products catalogue. Bedford, Millipore Corp., 82 p.
1976

O'Gower, A.K. and J.W. Wacasey, Animal communities associated with Thalassia diplanthera, and sand
1967 beds in Biscayne Bay. I. Analysis of communities in relation to water movements. Bull.Mar.Sci., 17:175-210

Odum, E.P., Fundamentals of ecology. Philadelphia, W.B. Saunders, 547 p. 3rd ed.
1971

Patrick, R., Biological measure of stream conditions. Sewage Ind.Wastes, 22:926-38
1950

Peres, J.M., Rapport du president, Comite du Benthos. Rapp.P.-V.Reun.CIESMM, 18(2):35-6
1965

_____, The Mediterranean benthos. Oceanogr.Mar.Biol., 5:449-533
1967

_____, La pollution des eaux marines. Paris, Gauthier-Villars, 230 p.
1976

Peres, J.M. and J. Picard, Manuel de bionomie benthique de la mer Mediterranee. Rec.Trav.Stn.Mar.
1958 Endoume, 23:1-154

Pielou, E.C., An introduction to mathematical ecology. New York, Wiley-Interscience, 286 p.
1969

Ranke, U., The sediments of the Gulf of Piran. Senckenb.Marit., 8:23-60
1976

Sanders, H.L., Benthic studies in Buzzards Bay. 3. The structure of the soft-bottom community.
1960 Limnol.Oceanogr., 5:138-53

_____, Marine benthic diversity: a comparative study. Am.Nat., 102:243-82
1968

Sanders, H.L., R.R. Hessler and G.R. Hampson, An introduction to the study of deep sea faunal
1965 assemblages along the Gay Head - Bermuda transect. Deep Sea Res., 12:845-67

Schlieper, C. (ed.), Research methods in marine biology. London, Sidgwick and Jackson, 356 p.
1972

SCOR-Unesco Working Group 17, Determination of photosynthetic pigments. Unesco Monogr.
1966 Oceanogr.Methodol., (1):1-36

Sournia, A. (ed.), Phytoplankton manual. Unesco Monogr.Oceanogr.Methodol., (6), 337 p.
1978

Stirn, J., Marine pollution in the Gulf of Trieste. Varstvo Narave, 3:137-84
1965

_____, Bioecenological methods for assessment of marine pollution and problems of indicator
1970 species. Paper presented to the FAO Technical Conference on marine pollution and its effects on living resources and fishing. Rome, Italy, 9-18 December 1970. Rome, FAO, FIRM:MP/70/E-41. Abstr. in FAO Fish.Rep., (99):p. 144 (1971)

Štirn, J., 1971 Ecological consequences of marine pollution. Rev.Int.Oceanogr.Med., 24:13-46

Štirn, J. et al., 1975 Selected biological methods for assessment of marine pollution. In Marine pollution and marine waste disposal, edited by E.A. Pearson and E. de Fraja Frangipane. Oxford, Pergamon Press, pp. 307-27

Strickland, J.D.H. and T.R. Parsons, 1968 A practical handbook of seawater analysis. Bull.Fish.Res.Board Can., (167):311 p.

Sverdrup, H.U., M.W. Johnson and R.H. Fleming, 1946 The oceans, their physics, chemistry and general biology. New York, Prentice Hall Inc., 1087 p.

Tranter, D.J. (ed.), 1968 Zooplankton sampling. Part 1. Unesco Monogr.Oceanogr.Methodol., (2):1-144

Uhlig, G., H. Thiel and J.S. Gray, 1973 The quantitative separation of meiofauna. Helgol.Wiss.Meeresunters., 25:173-95

Ursin, E., 1954 Efficiency of marine bottom samplers of the van Veen and Petersen types. Medd.Dan.Fisk-Havunders.(Ny Ser.), 1(7):1-7

Utermöhl, H., 1958 Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt.Int.Ver.Theor. Angew.Limnol., 9:38 p.

Vollenweider, R.A., 1974 A manual on methods for measuring primary production in aquatic environments. IBP Handb., (12):213 p.

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Part 1: Methods for detection, measurement and monitoring of water pollution. 1975. FAO Fish.Tech.Pap., (137):238 p.

Part 2: Guidelines for the use of biological accumulators in marine pollution monitoring, edited by J. Portmann. 1976, FAO Fish.Tech.Pap., (150):76 p.

Part 3: Sampling and Analyses of biological material. (Guidelines for the FAO(GFCM)/UNEP Joint Coordinated Project on Pollution in the Mediterranean), by M. Bernhard. 1976. FAO Fish.Tech.Pap., (158):124 p.

Echantillonage et analyse du matériel biologique. (Directives destinées au projet commun coordonné FAO(CGPM)/PNUE sur la pollution en Méditerranée), par M. Bernhard. 1977. FAO Doc.Tech.Pêches, (158):132 p.

Part 4: Bases for selecting biological tests to evaluate marine pollution. 1977. FAO Fish.Tech.Pap., (164):31 p.

Principes de sélection des tests biologiques pour l'évaluation de la pollution marine. 1981. FAO Doc.Tech.Pêches, (164):32 p.

Bases para la eleccion de ensayos biologicos para evaluar la contaminacion marina. 1981. FAO, Doc.Téc.Pesca, (164):34 p.

Part 5: Statistical tests, by F. Möller. 1979. FAO Fish.Tech.Pap., (182):131 p.

Part 6: Toxicity tests, by EG&G Bionomics, 1981. FAO Fish.Tech.Pap., (185), in preparation

Part 7: Selected bioassays for the Mediterranean. (Tests used by the FAO(GFCM)/UNEP Joint Coordinated Project on Pollution in the Mediterranean), by G. Bellan. 1981. FAO Fish.Tech.Pap., (208), in preparation

Bioessais sélectionnés pour la Méditerranée. (Tests utilisés dans le Projet commun coordonné FAO(CGPM)/PNUE sur la pollution en Méditerranée) par G. Bellan. 1981. FAO Doc.Tech.Pêches, (208), 37 p.

Part 8: Ecological assessment of pollution effects (Guidelines for the FAO(GFCM)/UNEP Joint Coordinated Project on Pollution in the Mediterranean), by J. Štirn. 1981. FAO Fish.Tech.Pap., (209), 70 p.

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